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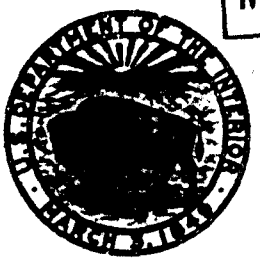
DEPARTMENT OF THE INTERIOR
BUREAU OF RECLAMATION

ANNUAL PROGRESS REPORT OF 1963 LABORATORY AND FIELD STUDIES ON AQUATIC WEEDS INVESTIGATIONS OF ALGAECIDAL AND HERBICIDAL MATERIALS AND AQUATIC ENVIRONMENTS

Report No. WC-13

Water Conservation Branch
DIVISION OF RESEARCH



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ABSTRACT

Progress of various laboratory and field studies concerned with submersed aquatic weed problems are covered. Results summarized are considered preliminary and not conclusive and generally require further investigation. Laboratory culture of filamentous algae and algaecidal evaluation techniques are described, including results of evaluation tests on compounds. Results of herbicidal evaluation tests of new herbicidal compounds and specification performance tests of aromatic solvent herbicides and emulsifiers are reported. Repeated applications of aromatic solvent treatments on pondweeds are shown to influence the growth and production of pondweed vegetative propagules. Tests on the influence of water quality on the herbicidal effectiveness of aquatic herbicide acrolein have shown negligible effects on pondweed response. Pondweeds grown and treated in both flowing and standing water combinations were studied from the standpoint of growth and herbicidal response to aromatic solvent treatments. Certain plants grown and treated in flowing water exhibited reduced injury to pondweeds as compared to that produced in standing water. Emulsifying agents commonly used for dispersing aromatic solvent aquatic weed killers produce greater emulsion stability in hard water than in soft water. Water temperature does not affect emulsion stability to any great extent. A pelletized herbicide prepared by adding a herbicide to a vinyl resin solution shows promise of having a longer period of effectiveness on sago pondweed than unpelletized material in an aquatic soil treatment. Techniques for on-site sampling of soil-water in canal soils and subsequent microanalytical analyses for oxygen content have been evaluated. Preliminary results of soil-water oxygen content were obtained from irrigation canal field study sites.

DESCRIPTORS--*algae/ *aquatic weeds/ *dispersing agents/ *water quality/ *emulsion/ *aromatic solvent/ *temperature/ *herbicides/ soil treatment/ toxicity/ weed control/ cultures/ *ecology/ dissolved oxygen/ *limnology/ biology/ aquatic life/ vinyl plastics/ irrigation O&M/ *chemical analysis/ plant/ growth/ botany

IDENTIFIERS--algaecidal and herbicidal evaluation/ emulsion stability/ pelletized herbicides

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INTRODUCTION

Laboratory and field investigations conducted on biological problems are often widely diverse and deal with many types of aquatic organisms found in the aquatic environmental complex of irrigation distribution systems. Many studies and tests are never carried beyond an exploratory state because of lack of potential indicated in preliminary results. This report covers annual progress made on various types of these studies conducted during 1963 as well as those investigations which are considered long term and are not sufficiently complete for conclusive interpretations.

Aquatic weed control field investigations covered in this report are conducted cooperatively with various Bureau regions.

Laboratory studies being reported are: algaecidal culture and evaluation techniques; results of preliminary evaluations of selected algaecides and aquatic herbicides; aromatic solvents and emulsifiers used to disperse these solvent herbicides; effects of contact herbicide applications on pondweed propagule production; certain exploratory tests dealing with aquatic weed response to herbicides applied in varying conditions of water quality; comparison of pondweed herbicidal injury produced in combinations of static and flowing water situations; influence of water hardness and temperature on the stability of emulsions produced by emulsifiers commonly used to disperse aromatic solvents; and the evaluation of laboratory prepared aquatic herbicide pellets for aquatic weed control through soil treatment.

1/Investigations of the Research Division, Bureau of Reclamation, U.S. Department of the Interior, in cooperation with the Crops Research Division, Agricultural Research Service, U.S. Department of Agriculture, Denver, Colorado.

Field studies reported are those concerned with certain aspects of the aquatic environment. Specifically, these concern the laboratory and field evaluation of a technique to sample and determine the oxygen content of interstitial water found in aquatic soils. Field evaluations of antifouling coatings for prevention of algae attachment to canal linings were continued during the 1963 irrigation season. These data are being reported in a separate laboratory report.

SUMMARY

A number of techniques for the laboratory culture of certain species of filamentous green algae have been evaluated. These included both alteration of the aquatic nutrient medium and physical environment. Culturing the algae in an inorganic nutrient solution under controlled temperature conditions of 70° F and 150-200 ft-c of cool-white fluorescent light on a 16-hour photoperiod was found to be most successful. These techniques are being used for the routine laboratory culture of filamentous green algae species known to infest water of irrigation distribution systems. These algae are used in laboratory studies of algaecidal compound evaluations and control measures.

Procedures have been developed for the routine evaluation of chemical compounds that may be useful in controlling or suppressing growth of filamentous green algae species in and on irrigation structures. Techniques for performing the tests are reported. The results of preliminary evaluations on 30 compounds are given, including chemicals that are known to possess good algaecidal properties as well as new compounds. A few of these newer compounds show some potential for use in controlling the filamentous green algae species tested.

Laboratory tests on the physical, chemical, and or biological character of aromatic solvent aquatic herbicides and emulsifying agents used to disperse these materials in irrigation water were conducted. The results of the evaluations of 12 aromatic solvent-type aquatic herbicides and 5 new emulsifiers are reported. Most of the solvent herbicides and 3 of the emulsifiers tested were considered suitable for use in controlling submersed aquatic weeds in irrigation systems.

A limited number of new herbicidal chemicals was evaluated for effectiveness in controlling rooted submersed aquatic weeds. A few of these materials showed some degree of herbicidal activity on the pondweeds tested, but were not considered as being particularly promising.

Studies on the effects of repeated aromatic solvent treatments on the growth and production of pondweed vegetative propagules were conducted. Results of these tests indicated that these contact herbicide treatments

significantly reduce the number and size of vegetative propagules produced by the rooted aquatic weeds. Two successive treatments were influential in causing continued reduced production of the vegetative propagule potential of these plant pests, but a third treatment did not further influence the production of propagule numbers.

Tests were made evaluating the influence of varying water quality on the herbicidal effectiveness of the aquatic herbicide acrolein on pondweed species. Results show that under laboratory test conditions differences in water hardness and alkalinities had little influence on the phytotoxicity of the compound.

Rooted pondweed species were cultured and treated under conditions of both flowing and standing water in a greenhouse study to evaluate the influence of water movement on the growth and herbicidal response of these weed plants to aromatic solvent treatments. Flowing water treatments were performed in a model test canal fabricated to provide continuous flowing water. Results of preliminary studies indicate that pondweeds grown in flowing water are less susceptible to injury from aromatic solvents than those grown in static water. Plants grown in static water showed less injury when treated in flowing water than those treated in standing water. These data show possible importance of treating pondweeds in a flowing water situation in herbicidal evaluation tests of aromatic solvent compounds for specification performance tests prior to field use.

A series of tests were conducted on four emulsifying agents in both soft and hard water and at four different water temperatures to determine the effects of water temperature and hardness on emulsion stability. In general, the emulsifiers produced considerably more stable emulsions in hard water, while the water temperature did not affect stability in most cases. One emulsifier at the 2 percent level produced very good emulsion stability in both soft and hard water and at all water temperatures.

An investigation was initiated to determine the merits of pelletized aquatic herbicide formulations prepared by mixing the herbicides into a vinyl resin solution for treatment of aquatic soils. In one series of tests, the results indicated that the pelletized formulation increased the period of herbicidal effectiveness. On the basis of these limited data, further studies are underway to fully evaluate the potentials of this resin for giving controlled release of the herbicide in aquatic soils for a more efficient utilization of the phytotoxicant.

A technique for on-site sampling of aquatic soil-water for subsequent microanalysis determinations of dissolved oxygen content has been evaluated and found to produce reliable results. Study of the soil-oxygen content of aquatic soils and its possible relationship to rooted aquatic

weed growth has been suggested by findings made from the field study of aquatic environments. A preliminary survey of interstitial oxygen in canal soils was made in irrigation canals of California and Colorado. Results of these tests indicate that soil oxygen is fairly high in the first 3 inches of a submersed canal soil. Present data are too preliminary to show the possible relationship between soil oxygen and pondweed growths. A detailed description of soil-water sampling techniques and the microanalytical method for dissolved oxygen content are given in the appendix of this report.

LABORATORY STUDIES

Laboratory Culture of Filamentous Green Algae for Use in Algaecidal Evaluations

Attached filamentous green algae present serious operational problems to water distribution systems. Extensive growths of attached algae filaments seriously reduce the capacity of concrete-lined canals and other irrigation structures. Colonies of the filamentous algae also create difficult problems in unlined irrigation canals by breaking loose from the attaching medium and clogging siphon tubes, fouling trash racks and pump inlets, and increasing water flow resistance by being caught on rooted submersed weeds. Because of the extensiveness of the algae problems on Bureau projects, a laboratory algaecidal evaluation program has been initiated as part of the overall weed control research program.

Laboratory evaluation of potential algaecides requires a reasonably uniform and continuing supply of plant material in which only a few typical algae species are represented. During the past year, emphasis has been placed on improving laboratory culture techniques for growing and maintaining these plants. Unlike the rooted vascular aquatic weeds, which are adapted to a wide range of growth conditions, many filamentous green algae species are quite specific in their environmental requirements. In general, filamentous green algae derive most of their required nutrients from inorganic sources, but laboratory experience has shown that some species apparently require either very specific physical environments or respond to some heterotrophic condition occurring in the environment.

The easiest approach to culture of filamentous algae is to merely use the soil-water culture medium, such as using algae growing in association with higher aquatic plants in greenhouse culture aquaria. A refinement of this technique is described by Pringsheim (1).* This source of filamentous

*Numbers in parentheses refer to literature cited at the end of the report.

green algae has been used occasionally in the laboratory, but found to be lacking in reliability for algaecidal evaluation purposes. Algaecidal test results are seldom reproducible when these cultures were used. The complication of multiple algae type associations makes interpretation of injury difficult.

To improve the quality of algae culture in the laboratory, a number of nutrient solutions and culture techniques were evaluated. Unfortunately most of the successful laboratory culture of algae described in the literature has been with either unicellular green or blue-green forms. Seldom are the filamentous green species referred to in investigations of algae. This may well be due to the difficulty in handling the masses of intertwined filamentous structures and possibly a complete lack of success in growing these filamentous algae under synthetic conditions.

A number of culture solutions and differing environments have been evaluated. Algae species used in these tests are those filamentous green types known to commonly exist in irrigation canals, including species that attach to solid substrate in flowing water. These cultures occasionally contain a few unicellular green and blue-green types as well as some filamentous blue greens. The difficulty and time required in producing and maintaining aseptic cultures do not appear warranted for these algaecidal evaluation tests. A mixture of species in these laboratory cultures would be more comparable to the complex algae species associations generally found in natural situations. The algae species used in these tests were primarily Oedogonium spp., Rhizoclonium spp., Cladophora spp., and occasionally Spirogyra spp. in mixed association with a limited representation of numerous other algal types, such as unidentified diatoms, Oscillatoria spp., Anabena spp., and Hydrodictyon spp.

One of the initial culture solutions used was that described by Krauss (2), which is a modification of Meyers nutrient solution utilized for the culture of Chlorella. Filamentous green algae grown in this nutrient medium exhibited considerable variability in rate of growth and periods of survival.

Physical conditions of the culture environment were altered a number of ways in attempts to improve the growth rates when using the modified Meyers nutrient solution. This included laboratory ambient temperatures ranging from 70° to 90° F, as well as constant temperatures of 60° and 70° F in a growth chamber. Cool-white fluorescent illumination was varied from approximately 50 ft-c to 700 ft-c on a 16-hour photoperiod. Aeration of the culture solutions was attempted using low volumes of compressed air, bubbled through the water contained in 1-quart or 1-gallon glass culture vessels. Pure compressed carbon dioxide was also metered into the culture solutions in certain tests. The evaluation of

success of a culture environment was generally limited to empirical observation of growth rate and longevity of a healthy green culture.

Observations of results indicated that constant temperatures of 70° F under approximately 100 ft-c of cool-white fluorescent illumination for a 16-hour photoperiod produced optimum conditions. Bubbling of air or CO_2 into the culture vessel appeared to do little to improve culture growth. A considerable decline in solution pH was observed when using CO_2, producing pH values ranging from 5.8 to 6.2. When CO_2 was added intermittently, the pH was lowered to 4.9. Carbon dioxide was metered into the solutions at the rate of about 5.4 liters per hour. Gummert et al (3) reported the use of a 1-percent CO_2-air mixture at the rate of about 550 liters per hour in mass algae culture vessels. At this rate the CO_2-air mixture would apply carbon dioxide at a rate comparable to that introduced in the above tests. Various other authors report that a 5-percent mixture of CO_2 and air are optimum and that toxic symptoms have been observed when pure CO_2 is introduced directly into the nutrient solution. These CO_2-air mixtures have not been evaluated to date in Denver laboratory tests.

Successful algae cultures appear to proceed into a log phase of growth soon after being placed in nutrient solutions. Considerable variability between cultures has been observed, suggesting the possible need for additional chemical or organic complexes that might occur in natural situations. Cultures normally developed within about 3 to 6 weeks to fill the top one-third of a culture vessel and then growth rates subside.

Machlis (4) reported that certain Oedogonium species could be cultured more efficiently by the addition of vitamin B12 and possibly an unknown growth factor obtained from a water extraction of autoclaved topsoil. Various nutrient solutions were used by Machlis in this paper. A preliminary evaluation of Machlis' techniques was tried using the modified Meyer nutrient solution described earlier. The suggested growth factor was prepared by autoclaving approximately 1,000 grams of locally available topsoil covered with 1,500 ml of distilled water for 15 minutes at 121° C and 15 pounds pressure. The extract was then decanted and filtered through a bacteriological filter. Culture containers were 12-liter plastic baskets inoculated with a laboratory culture of Oedogonium. Very little improvement in the cultures was observed by the addition of the soil extract. Probably the soil that was used by Machlis contained some compound or micro-organism that did not occur in the soil used in our tests. Cultures grown in solutions containing vitamin B12 did produce slight improvements in growth rates.

The most successful nutrient solution that has been evaluated for the culture of Oedogonium spp., Rhizoclonium spp., Cladophora spp. and Hydrodictyon spp. is one used by the U.S. Public Health Service at the

Taft Engineering Center, Cincinnati, Ohio (5). This is a modification of Chu's No. 10 nutrient medium. The formulation of this solution is given in Table 1.

Table 1

NUTRIENT SOLUTION USED FOR LABORATORY CULTURE OF FILAMENTOUS GREEN ALGAE

Chemical	Grams/500 ml water for stock solutions
$Ca(NO_3)_2 \cdot 4H_2O$	2.9
K_2HPO_4	0.5
$MgSO_4 \cdot 7H_2O$	1.25
Na_2CO_3	1.00
$Na_2SiO_3 \cdot 5H_2O$	2.20
Ferric citrate	0.175*
Citric acid	0.175*

*Made up as one solution and refrigerated when stored.

Nutrient solutions for algae culturing are made by using 10 ml of each stock solution, made up to 1 liter in laboratory tapwater. The pH of these solutions ranges from 9 to 10. A constant pH of 8 can be maintained by the use of the buffer Tris (hydroxymethyl) aminomethane (pH 7-9) at the rate of 1 gm/liter of nutrient solution.

Laboratory tests were established to evaluate this nutrient solution under various environmental conditions, pH ranges, and with various additives. The soil extract additive was prepared and added at the same concentrations as previously discussed. Vitamin B12 was included in certain combinations at the rate of 1 part per billion (ppb) v/v with the nutrient solution. The lower pH values were obtained by adding 0.996 gms of K_2HPO_4 and 0.530 gms of KH_2PO_4 to each liter of nutrient solution. Results of these tests are given in Table 2.

Cultures grown in the nutrient solutions with lower pH values from 7.5 to 7.8 did not develop well and in some instances toxicity was indicated. A solution pH of 9.5 appeared to be optimum for these species under test conditions used. There did not appear to be a great difference between cultures grown in constant temperature of 70° F and the one with temperatures ranging from 68° to 82° F. However, these cultures were maintained for a few weeks after the observation date and ambient temperatures exceeded 85° F. At this time, all cultures exhibited rapid decline in vigor. The addition of soil extract growth factor and vitamin B12 did

Table 2

RESULTS OF CULTURING *Oedogonium* spp. IN MODIFIED CHU NO. 10 INORGANIC NUTRIENT SOLUTION WITH VITAMIN B12 AND SOIL EXTRACT ADDITIVES UNDER VARIOUS CONDITIONS OF TEMPERATURE AND pH RANGES
Replicated Tests Made in 1-quart Glass Culture Vessels

Culture solution	pH of culture solution	Culture temperature, °F and aquaria location	Ft-c of cool-white fluorescent: 16-hr photoperiod	Condition of algae cultures at the end of a 3-week period
Inorganic nutrient solution in tapwater	9.5	68-82°, grown on laboratory bench	150-250	Culture densely green, continued filament growth
Inorganic nutrient solution and soil extract in tapwater	9.3-9.5	68-82°, grown on laboratory bench	150-250	Culture densely green, continued filament growth
Inorganic nutrient solution, soil extract, and 1 ppb v/v of vitamin B12 in tapwater	9.3-9.5	68-82°, grown on laboratory bench	150-250	Culture densely green, continued filament growth
Inorganic nutrient solution and 1 ppb v/v of vitamin B12 in tapwater	9.5	68-82°, grown on laboratory bench	150-250	Culture densely green, extensive amount of filament growth
Inorganic nutrient solution in tapwater	9.5	70°, continuous in controlled growth chamber	150-250	Culture densely green, continued filament growth
Inorganic nutrient solution and soil extract in tapwater	9.3-9.5	70°, continuous in controlled growth chamber	150-250	Culture densely green, continued filament growth

Table 2--Continued

Culture solution	pH of culture solution	Culture temperature, °F and aquaria location	Ft-c of cool-white fluorescent 16-hr photoperiod	Condition of algae cultures at the end of a 3-week period
Inorganic nutrient solution, soil extract, and 1 ppb v/v of vitamin B12 in tapwater	9.3-9.5	70°, continuous in controlled growth chamber	150-250	Culture densely green, limited amount of filament growth
Inorganic nutrient solution and 1 ppb v/v of vitamin B12	9.5	70°, continuous in controlled growth chamber	150-250	Culture densely green, limited amount of filament growth
Inorganic nutrient solution in tapwater	7.6	68-82°, grown on laboratory bench	200-300	Culture showing considerable chlorosis and filaments disintegrating
Inorganic nutrient solution and soil extract in tapwater	7.7-7.8	68-82°, grown on laboratory bench	200-300	Culture green, very limited filament growth
Inorganic nutrient solution, soil extract, and 1 ppb v/v of vitamin B12 in tapwater	7.7-7.8	68-82°, grown on laboratory bench	200-300	Culture green, moderate amount of filament growth
Inorganic nutrient solution and 1 ppb v/v of vitamin B12 in tapwater	7.5	68-82°, grown on laboratory bench	200-300	Culture showing chlorosis and filaments disintegrating

in certain instances slightly improve the growth of the filaments, but not sufficiently greater to warrant routine use of these additives to the inorganic nutrient medium.

Recently tests were made by culturing the previously mentioned algae in the modified Chu nutrient solution at constant temperature of 60° F. These cultures all remained healthy and densely green, but filament growth was very slow.

Results of these studies indicate that the filamentous green algae species tested grew best in the modified Chu No. 10 nutrient solution made up in tapwater and placed in an environment of 150 to 200 ft-c of cool-white fluorescent light at 70° to 80° F. These conditions have been selected for routine culture of algae for algaecidal evaluation. Experience has shown that healthy cultures can be maintained up to 4 months in the initial nutrient solution by occasional addition of 20 ml of calcium nitrate stock solution to the 1-gallon glass culture vessel, after which time the nutrient solution must be replaced.

Further investigation will be continued in an attempt to improve the rate of algae cell division. Occasionally a culture will suddenly decline and die. Also, considerable variability has been noted in the rate at which various cultures develop and the total time they remain in the log-growth phase. Reasons for these differences are not known, but assumed to be associated with genotype and/or phenotype plant specimens or changes in availability and uptake of nutrients, either inorganic or organic.

A rather unique method for culture of attached filamentous green algae was suggested from investigations of Whitford and Schumacher (6) regarding current effect on the mineral uptake of Oedogonium.

To evaluate the potential of laboratory culture of certain filamentous green algae known to attach to solid substrates, a small model recirculating system was devised. A 20-liter plastic container was fixed with an outlet tube at the bottom. This served as a reservoir for the modified Chu No. 10 nutrient solution. A small, 0.75-gpm pump was arranged to recirculate nutrient solution from the reservoir through a shallow plastic channel 2-1/2 feet long and 2 inches in width. Flow volume averaged 2.7 liters per minute with a free flow surface velocity of 5 cm per second. This small channel was arranged to receive 100 ft-c of cool-white illumination and ambient temperatures of 66° to 70° F. The nutrient stock was inoculated with filaments of a laboratory grown culture of Rhizoclonium spp. In the initial tests, water temperatures increased to above 85° F under continuous operation. During this period, a heavy growth of filamentous blue-green algae developed on the channel and in the reservoir. To maintain culture solution temperatures nearer to a 65° to 70° F range, a stainless steel coil was immersed in the

reservoir and cold tapwater recirculated through it. This maintained a temperature of 64° F. A few days after replacement of the nutrient solution and reinoculation with Rhizoclonium filaments, a very dense growth of attached algae developed in the channel. This growth was maintained for approximately 8 weeks. Normally in standing water culture, Rhizoclonium has not been observed to develop holdfast cells and attach to solid substrates.

This technique certainly appears promising for laboratory culture of attached filamentous green algae specimens for small-scale studies. This test apparently demonstrated that development of Rhizoclonium holdfast cells and rather rapid filament development can be enhanced by flowing water. Future investigations on growing attached filamentous green algae for specific algaecidal study purposes appear justified and will be conducted as time permits.

Algaecidal Evaluation Techniques Presently Used and Results of Some Typical Algaecidal Tests

A few techniques for evaluating algaecides have been reported and were reviewed (7) (8) (9). Only a very limited amount of the techniques described appeared to be directly adaptable for use in evaluating compounds on filamentous green algae. Although all of these methods are basically standing water beaker tests, considerable modification of the described techniques have been found necessary.

The presently used procedure for evaluating the potential activity of a candidate algaecide consists of treating a small mass of Oedogonium or Rhizoclonium filaments in a standing water test. This mass of algal filaments is estimated by volume to equal 0.5 to 1.0 gram fresh weight. A number of weighings were made to determine the relative fresh weight of filaments selected for a given treatment. These estimated amounts were found to correspond to this weight range. In routine algaecidal evaluation, algae sample sizes are estimated.

Algae cultures used in evaluation tests are laboratory grown in an inorganic nutrient solution under temperature regimes of 60° to 70° F and 150 to 200 ft-c of cool-white fluorescent illumination. Algae cultures are usually mixed species of Oedogonium or Rhizoclonium growing in association with occasional unicellular green specimens and filamentous blue-green algae.

Aqueous solutions of the algaecidal compounds are made up in concentrations of 10, 5, 1, 0.5, 0.25, 0.1, and 0.05 parts per million (ppm) of active ingredient in initial tests. Subsequent tests often include the range of treatment concentrations, but with reduced number of individual test concentrations.

Algal filaments taken from the culture medium are exposed to the algaecidal concentrations for a period of 60 minutes, then removed and rinsed in running tapwater for three cycles of container filling and draining. The treated algae specimens are then held in tapwater-filled quart jars for observation and injury rating. Treatment solution, rinse water and holding water temperatures are all held to a tolerance of 70° to 75° F. During periods in the summer months when tapwater chlorination is high, it was found necessary to limit the direct use of running tapwater. Rinse water during these periods is dechlorinated by agitation and followed by a period of standing in an open container for 2 to 4 hours.

Transfer of algae specimens from an inorganic nutrient medium to tapwater might be considered objectionable and cause toxicity or decline symptoms. This factor was considered and has been evaluated on numerous occasions. Generally there have been no noticeable differences exhibited in these tests. In all cases of algaecidal compound evaluation, an untreated check specimen is exposed to all treatment conditions and held for observation. The numerical injury rating or decline observation exhibited by check specimens is deducted from that exhibited on treated algae.

The relative size of the plant sample to be treated in relation to the treatment solution volume has been reported to have an influence on the amount of injury (10). The influence of plant sample size in the 500-ml treatment solution has been evaluated and results observed on occasions when plant sample sizes were not closely determined. Some differences were noted when excessive amounts of algae were treated. Consequently the plant sample size is regulated, as previously indicated.

Because of the variability in plant material cultured in the manner previously described, a number of genotypical and/or phenotypical variations can occur. These differences may alter the response of algae specimens to a treatment. To overcome this potential hazard in the rating of a number of algaecides, a standard treatment is used in all evaluation tests of unknown compounds. Copper sulfate is utilized as a standard algaecide for injury comparisons of a treatment series. Any one evaluation test showing excessively high or low activity on the algae treated with standard copper sulfate concentrations is considered atypical, and the results of unknown compounds are not considered representative and cannot be compared with other materials. Tests giving these results are repeated using a different source of algae.

At present an observational injury rating system based on a 0 to 10 scale is used to evaluate a candidate compound. While this rating system has some recognized limitations, it has been found to be suitable for the present algaecidal evaluation program.

When unicellular algal suspensions are utilized in chemical phytoxicity studies, the changes in growth and survival of cells is often determined by changes in optical density of the cells in the culture medium. Intact filamentous green algae would not be adaptable to optical density determinations as a criteria of colony size without separation of individual cells from the filamentous colony. Separation of the individual one-celled plants from their position in a filament would probably produce an atypical response to algaecidal chemicals. This type of evaluation would be best suited to determining growth suppression data on plants treated. A more desirable algaecide for flowing water is one that is rapidly absorbed and will cause rapid death of the plant cells. Growth suppression evaluation would be important to some aspects of algae control in irrigation systems where the toxicant is applied to a solid substrate as an attachment inhibitor.

Another approach for evaluating algaecidal injury consisted of a chlorophyll extraction of treated algae. A standard acetone extraction was used (11) to produce the chlorophyll solution. Chlorophyll (a and b) concentrations of the extract were determined photometrically using a red tube on the spectrophotometer and peak transmittance determined at wave lengths of 658 and 642.5 millimicrons. Comparative chlorophyll solutions were made by acetone extractions of untreated Oedogonium filaments. Ether dilutions were made up from these extractions and a standard curve developed from the varying degree of transmittance by changes in chlorophyll concentration. Treated plant extractions were compared to this standard curve in an attempt to correlate degree of injury with changes in chlorophyll content.

Results of this study indicated very little correlation between extracted chlorophyll density and observations of injury and subsequent survival. A number of variables contributed to the wide differences experienced. First and foremost was in establishing a standard chlorophyll content of the algae species on a basis of milligrams of chlorophyll per grams dry weight of plant cells. Extractions of various samples showed differences that made it difficult to establish any comparative standard. Some difficulty was also experienced in making sure of complete chlorophyll extraction of the small plant samples used. Another critical condition for the extractions of treated algae would be the holding period following treatment. A slow acting or possibly a systemic algaecide might not produce effects on chlorophyll content as rapidly as contact type algaecides. Any uniform time of chlorophyll extraction might indicate high chlorophyll content and limited injury of a compound, while in reality it may be a slow acting compound producing maximum injury.

Certain other approaches to establishing a reliable measurement of chemical injury to algae have been evaluated with only limited success resulting. One obvious technique is to determine possible differences in dry weight of severely injured and untreated algae. Little correlation appeared

between degree of injury and changes in dry weight over the 2-week observation period.

Although the observational rating system at present appears to be the most reliable method of determining injury to treated algae, it has limitations. Probably the most critical question is whether or not one is still rating the same algal cells that were treated, because of the rapid cell division that can take place in these type plants. This would be especially true when lower injury values of 5 or less are observed and when limited growth inhibition is exhibited. The present feeling is that compounds that produce less than near total kill (injury ratings of 8-10) would not be considered as being particularly promising and would probably be rejected as an algaecide for use in water conveyance systems.

Results of Algaecidal Evaluation Tests on Selected Compounds

A number of compounds have been preliminarily tested using the most suitable culture and evaluation techniques described in this report. Injury is based on the 0 to 10 scale, described as follows: 0 = no injury; 1, 2, 3 = slight injury, as evidenced by some bleaching; 4, 5, 6 = moderate injury, some cell division; 7, 8, 9 = severe injury; 10 = complete kill of initial culture. The results of these tests are listed in Table 3.

The mean activity ratings given in Table 3 were obtained by determining the average injury rating obtained at all chemical concentrations over the 2-week observation period. This average activity rating provides some index for comparing chemicals.

A number of the more promising compounds were ranked according to highest mean activity ratings and are listed accordingly in Table 4. Statistical analysis for significance in ranking would not be justified because these are preliminary tests and the data are useful mainly in eliminating compounds that show limited activity.

Emulsified aromatic solvent was included in these tests to determine the minimum lethal concentrations on typical filamentous green algae species. This aquatic herbicide has on occasions been used as an algaecide in irrigation distribution systems.

Table 3

RESULTS OF PRELIMINARY EVALUATION OF SELECTED COMPOUNDS FOR ALGAECIDAL ACTIVITY ON MIXED CULTURES OF THE FILAMENTOUS GREEN ALGAE Rhizoclonium spp. AND/OR Oedogonium spp.

Laboratory No.	Algaecidal compound	Weekly injury ratings obtained at a given concentration of chemical in ppm of active ingredient												Mean activity rating1/
		1 week						2 weeks						
		5.0	1.0	0.5	0.25	0.1	0.05	5.0	1.0	0.5	0.25	0.1	0.05	
--	Copper sulfate, conc. as copper2/	9.4	8.4	6.6	4.2	2.6	2.0	9.9	9.1	6.9	4.8	2.6	2.0	5.7
--	Zinc sulfate, conc. as zinc	5	2	1	0	0	0	4	2	0	0	0	0	1.2
--	Potassium permanganate	0	0	0	0	0	0	1	0	0	0	0	0	0.1
--	Sodium hypochlorite	10	5	8	6	2	1	10	6	7	5	2	1	5.3
574	Copper chelate	3	0	0	0	0	0	4	0	0	0	0	0	0.6
582	Copper chelate	0	0	0	0	0	0	0	0	0	0	0	0	0
788	Copper sulfate, sodium citrate mixture	5	0	0	0	0	0	6	1	0.5	0	0	0	1.0
753	bis (tri-n-butyltin) oxide with solubilizer	9	7	6	4	4	3	10	10	9	7	7	7	6.9
751	Tributyltin chloride with solubilizer	10	10	10	10	8	8	10	10	10	10	7	5	9.0
700	Copper methanearsonate	6	4	3	5	0	0	10	7	4	6	0	0	3.4
701	Silver methanearsonate	10	10	10	9	7	6	10	10	10	8	5	4	8.3
856	Bromine salt of tris- (1-dodecyl-3-methyl-2-phenyl-benzimidazolium) ferricyanide	6	4	1	0	0	0	2	0	0	0	0	0	1.3
855	Tris- (1-dodecyl-3-methyl-2-phenylbenzimidazolium) ferricyanide	7	2	0	0	0	0	8	0	0	0	0	0	1.3
655	2-amino-3-chloro 1,4 naphthoquinone	4	0	0	0	0	0	7	2	1	0	0	0	1.2
462	2,3, dichloro 1,4 naphthoquinone	7	7	5	0	0	0	8	8	5	0	0	0	3.3
391	Rosin amine D acetate	8	8	6	2	2	2	9	9	6	2	2	2	4.8
622	Di-isobutyl phenoxy ethoxy ethyl dimethyl benzyl ammonium chloride	3	1	0	0	0	0	5	2	1	1	1	1	1.3

Table 3--Continued

Laboratory No.	Algaecidal compound	Weekly injury ratings obtained at a given concentration of chemical in ppm of active ingredient												Mean activity rating1/
		1 week						2 weeks						
		5.0	1.0	0.5	0.25	0.1	0.05	5.0	1.0	0.5	0.25	0.1	0.05	
757	1:1'-ethylene-2:2'-dipyridylium dibromide cation	8	5	1	1	1	1	10	7	3	3	3	1	3.7
758	1,1'-dimethyl-4,4'-dipyridylium cation	6	6	6	1	1	1	9	8	8	5	3	3	4.8
858	Monocottonseed trimethyl Quaternary ammonium chloride	7	2	1	0	0	0	7	1	1	1	1	1	1.8
859	Monocottonseed and dicoco Quaternary ammonium chlorides	5	0	0	0	0	0	0	0	0	0	0	0	0.4
860	Monotallow and dicoco Quaternary ammonium chlorides	0	0	0	0	0	0	0	0	0	0	0	0	0
861	Monolauryl trimethyl Quaternary ammonium chloride	0	0	0	0	0	0	1	1	1	1	1	1	0.5
820	3-p-chlorophenyl 1,1 dimethylurea	1	0	0	0	0	0	1	1	0	0	0	0	0.3
818	Sodium cis-3-chloro-acrylate	2	2	1	1	1	1	1	1	0	0	0	0	0.8
--	Sodium methyl dithiocarbamate	3	1	0	0	0	0	3	1	0	0	0	0	0.7
849	4-amino-3,5,6-trichloro picolinic acid	0	0	0	0	0	0	0	0	0	0	0	0	0
825	Coco diamine	7	2	1	1	1	1	9	2	1	1	1	1	2.3
816	Acrolein acrylaldehyde	9	8	4	2	1	1	10	7	5	3	1	1	4.4
		150 ppm	100 ppm	80 ppm	50 ppm	10 ppm	1 ppm	150 ppm	100 ppm	80 ppm	50 ppm	10 ppm	1 ppm	
	Industrial grade xylene dispersed with 2% emulsifier	10	3	0	1	0	0	10	5	3	2	0	0	

1/Mean activity rating for a given compound is the average of combined injury ratings at all concentrations over the 2-week observation period.
2/Injury for copper sulfate is means of eight separate evaluation tests.

Table 4

COMPARISON OF COMPOUNDS EXHIBITING HIGHEST ALGAECIDAL ACTIVITY ON THE FILAMENTOUS GREEN ALGAE *Rhizoclonium* AND *Oedogonium*

Laboratory No.	Algaecidal compound	Mean activity rating number
751	Tributyltin chloride	9.0
701	Silver methanearsonate	8.3
753	Bis (tri-n-butyltin) oxide	6.9
—	Copper sulfate	5.7
—	Sodium hypochlorite	5.3
391	Rosin amine D acetate	4.8
758	1,1'-dimethyl-4,4'-dipyridylium cation	4.8
816	Acrolein	4.4
757	1:1'-ethylene-2:2'-dipyridylium dibromide cation	3.7

Most of the more active compounds listed in Table 4 have been used successfully in some form as algaecides. Copper sulfate, of course, is used extensively because of cost, ease of application and relatively low fish and other animal toxicity. Some of the other compounds do exhibit sufficient activity to warrant further investigation for special applications, such as additives to antifouling coatings on concrete canal linings. One of these compounds (Number 753) is presently under test as the active ingredient in an antifouling paint.

A potential list of chemicals that might possess algaecidal activity would be extensive and only a few have been included in the present tests. The results of the compounds tested to date do, however, indicate the usefulness of the presently used algae culture and algaecidal evaluation procedures.

Laboratory Evaluation of Emulsifiers Used to Disperse Aromatic Solvent Aquatic Herbicides

Emulsifier manufacturers continually develop new and improved emulsifiers for agricultural pesticide chemicals. Many of these materials are submitted to the Denver laboratory for evaluation of suitability for use in dispersing aromatic-solvent aquatic herbicides for pondweed control. A number of these compounds are scrutinized and tested annually in the laboratory. Materials performing well in laboratory tests are suggested for further testing in the field by Bureau project offices. Development of suitable laboratory tests and evaluation of new materials have aided in developing the use of more efficient emulsifiers. This has resulted in better aquatic weed control with a considerable reduction in cost of emulsifiers by allowing for a significant reduction in percent emulsifier required to adequately disperse the solvent herbicide in irrigation water.

The laboratory test method used to evaluate emulsifiers is that developed in the Denver laboratory and described in Chemical Engineering Laboratory Report No. W-1 (12).

Results of the five selected emulsifiers tested in the laboratory during the past year are listed in Table 5. The suitability of the emulsifiers to disperse solvent herbicides is based on the premise of the stability of the oil-water emulsion produced. The index of this stability is based on the amount of cream layer and/or oil that separates from the water medium during a given period of standing. These oil-cream separations are indicated in the test results of Table 5 and relate the potential usefulness of the emulsifying compound.

Table 5

RESULTS OF LABORATORY EVALUATION OF EMULSIFIERS PROPOSED FOR USE IN DISPERSING AROMATIC SOLVENT HERBICIDES

Laboratory Sample No.	Percent emulsifier by volume with xylene	Mean results of two test replications — Divisions of cream and oil separation after standing for 1, 2, and 4 hours						Suitability rating at a given emulsifier conc.
		1 hour		2 hours		4 hours		
		Cream	Oil	Cream	Oil	Cream	Oil	
850	1.0	5.0	0	5.5	0	17.5	1.0	Poor
	1.5	1.5	0	2.5	0	3.5	Trace	Good
	2.0	Trace	0	1	0	1.5	Trace	Excellent
851	1.0	7.0	8.0	3.0	11.0	2.0	13.0	Poor
	1.5	14.0	12.0	14.0	11.0	8.5	14.5	Poor
	2.0	16.0	7.0	14.5	9.5	10.5	13.0	Poor
852	1.0	27.0	1.5	27.5	1.5	27.5	2.0	Poor
	1.5	23.0	2.0	22.0	3.5	20.0	5.5	Poor
	2.0	26.0	0	28.5	0	28.0	1.0	Poor
863	1.0	2.0	0	3.0	0	3.5	1	Good
	1.5	Trace	0	1	0	1	Trace	Excellent
	2.0	0	0	Trace	0	Trace	0	Excellent
869	1.0	5.0	1.0	9.0	1.5	14	2.0	Fair
	1.5	Trace	0	2.0	0	4.0	0	Good
	2.0	Not tested at this concentration						

Emulsifying compounds 850, 863, and 869 would be considered suitable for dispersing aromatic solvent aquatic herbicides. Emulsifier No. 863 should produce good results down to the 1 percent level. The other two would be questionable when used below the 1.5 percent level.

Evaluation of Aromatic Solvents for Use By Bureau of Reclamation Projects and Cooperating Irrigation Districts

Samples of xylene and aromatic solvents were received for testing for suitability as aquatic herbicides pursuant to requests from regional and project offices. These samples were analyzed for conformance to physical and chemical requirements and tested for herbicidal activity on three species of submersed aquatic weeds. These tests were performed for the purpose of obtaining data useful to regional and project offices in selecting suitable aquatic herbicides, and provide information useful for further development and improvement of specifications and requirements for this type of aquatic herbicide.

The samples were tested for conformance to physical and chemical requirements listed in the tentative specifications included in Chemical Engineering Laboratory Report No. SI-17(13). The results of the test for aromaticity of samples are tabulated in Table 6.

Table 6

ANALYSES OF AROMATIC SOLVENTS AND XYLENE SAMPLES FOR HYDROCARBON TYPES BY ASTM: DESIGNATION 1319

Laboratory: Hydrocarbon type (percent by volume)			
Sample No.	Saturates	Olefins	Aromatics
831	4.1	0	95.9
832	1.2	0	98.8
833	0.2	0	99.8
834	4.2	0	95.8
835	9.7	0	90.3
836	0.2	0	99.8
837	8.9	0	91.1
838	14.0	0	86.0
839	0.7	0	99.3
842	0.6	0	99.4
844	1.1	0	98.9
853	0.9	0	99.1
854	0.6	0	99.4

All samples tested had an aromatic content greater than the minimum requirement of 85 percent.

Distillation range tests of aromatic solvents for conformance to physical requirements for the samples are included in Table 7.

Table 7

RESULTS OF DISTILLATION RANGE TESTS OF AROMATIC SOLVENTS FOR CONFORMANCE TO PHYSICAL REQUIREMENTS

Specified requirements	Flash point °F	Distillation range ASTM D86-54, degrees F at 760 mm Hg pressure: Initial boiling point	Temperature at which percent distillate by volume was recovered: 10%	50%	90%	End point	Percent water
Laboratory Sample No.	80° minimum	240° minimum	Greater than 265°	Less than 320°	Less than 380°	420° maximum	0.2% maximum
832	97	304	317	*331	372	*422	Nil
834	91	276	284	292	308	312	Nil
835	88	272	282	291	331	357	Nil
838	99	310	319	**325	333	343	Nil
842	93	274	293	313	339	354	Nil

*Samples did not meet specified requirement.
**Sample exhibited a slight deviation from specified requirement.

The results of distillation range tests of xylene samples for conformance to specified requirements are listed in Table 8.

Each sample of solvent was subjected to a herbicidal activity test by treating potted cultures of sago pondweed, Potamogeton pectinatus, American pondweed, P. nodosus, and waterweed, Elodea canadensis, in a flowing water situation. Details of this greenhouse culture and herbicidal evaluation technique are similar to that described by Frank, Otto and Bartley (14).

The flowing water test is conducted in a small flume where treatment water is recirculated at a volume of 0.166 cfs and a surface velocity of 0.63 fps. The herbicidal solvent is dispersed in the treatment water with an anionic-nonionic surfactant (Laboratory Sample No. 755) which is used at a rate of 1.5 percent by volume of solvent.

Replicated potted cultures of each species are treated at herbicidal concentrations of 200 and 600 parts per million (ppm) active ingredient (a.i.). The plants are exposed to the recirculated herbicidal solution for a period of 30 minutes, then removed to a 20-liter aquaria containing clean tapwater for rinsing. The treated plants are held in the rinse container for about 30 minutes and then placed in a 20-liter glass jug for a 3-week period of injury observation.

Table 8

RESULTS OF DISTILLATION RANGE TESTS OF XYLENES FOR CONFORMANCE TO PHYSICAL REQUIREMENTS

Specified requirements	Flash point °F	Distillation range ASTM D 86-54, degrees F at 760 mm Hg pressure: Initial boiling point	Temperature at which percent distillate by volume was recovered: 5%	90%	End point	Percent water	Specific gravity at 60°/70° F
			Greater than	Less than			
Laboratory Sample No.	75° minimum	253° minimum	266°	293°	311° maximum	0.2 maximum	0.850 min. 0.870 max.
831	83	272	273	276	276	Nil	0.855
833	83	272	274	278	282	Nil	0.859
836	80	264	274	278	280	Nil	0.861
837	78	268	270	276	280	Nil	0.858
839	79	271	275	279	285	Nil	0.864
844	79	*240	270	279	281	Nil	0.859
853	84	269	275	**300	310	Nil	0.867
854	80	271	273	277	281	Nil	0.869

*Sample did not meet specified requirements.
**Sample exhibited a slight deviation from specified requirements, but considered acceptable for use as an aquatic herbicide.

Water temperature during plant culture, treatment, rinse, and post-treatment observation is controlled in a range of 65 to 75° F.

Herbicidal injury ratings used are similar to those described by Frank et al (14) with some slight modifications that are used to rate contact herbicides. In general, the maximum attainable injury rating for aromatic solvents seldom exceeds 8 on the 0 to 10 scale, where 0 = no injury and 10 = complete kill without regrowth. Also, injury by contact herbicides is limited to above ground plant parts only, whereas with some systemic-type herbicides the injury ratings may include effects on root and rhizome tissue.

The herbicidal activity rating scale used is described as follows:

0 - No apparent injury.
1-2-3 - Slight injury.
4-5-6 - Moderate injury.
6-7-8 - Severe injury, but with some regrowth at the end of the 3-week observation period. The amount and vigor of regrowth is reflected in these final 3-week ratings.

*10 - Total kill of all plant material without regrowth.

*This degree of injury has never been attained with aromatic solvent treatments in greenhouse tests.

The results of herbicidal activity tests on solvent samples described in this report are summarized in Table 9. The samples of aromatic solvents and xylenes tested during the past year met all specified requirements, except for slight deviations of Samples No. 832, 838, and 853. Sample No. 832 was rejected because of the two failures of requirements of physical tests. All other samples were considered acceptable. The herbicidal activity ratings of all materials tested were quite similar.

Aromatic solvents submitted for acceptance tests for use by Bureau of Reclamation projects or cooperating irrigation districts have continually improved over the years. The past year's evaluation of these materials illustrates the increasing uniformity of this type of aquatic herbicide.

Evaluation of Selected Herbicidal Compounds on Rooted Submersed Aquatic Weeds

A limited number of chemical compounds have been evaluated for activity on submersed aquatic weeds during the past year. These materials were either submitted to the Bureau laboratories by manufacturers for evaluation of their algaecidal and/or herbicidal potential or may be materials that are suggested for specific herbicidal tests from other areas of research being conducted in the laboratory or the field.

These materials are subjected to herbicidal activity tests by treating greenhouse grown cultures of sago pondweed, Potamogeton pectinatus, American pondweed, P. nodosus, and Elodea canadensis. Details of culture and evaluation procedures are those described by Frank, et al (14). Generally, the potted cultures of the previously indicated aquatic weeds are exposed to the candidate compounds in 20-liter aquaria maintained in one of two greenhouses. The test sequence usually follows the pattern of a preliminary continuous contact test, followed by tests with reduced exposure time, and may be finally tested in a flowing water situation. Test methods, although basically similar, are modified to fit the specific type of material. Likewise the concentration of the material under test may be varied as knowledge of the material may suggest.

Herbicidal injury to the treated plants is that described by Frank, et al (14) with modifications that were described in the previous section in this report on evaluations of aromatic solvent-type herbicides. Results of the herbicidal activity tests of each compound are tabulated in the following tables.

Table 9

HERBICIDAL ACTIVITY OF SAMPLES ON SUBMERSED AQUATIC WEEDS

Laboratory Sample No.	Solvent concentration ppm	Injury scale ratings/ E. canadensis	P. nodosus	P. pectinatus	Activity Index No. b/
831	200	3.7	3.0	3.0	3.2
	600	4.7	4.7	4.7	4.7
832	200	4.0	3.7	3.0	3.6
	600	6.3	6.0	5.3	5.9
833	200	3.3	3.7	3.7	3.6
	600	5.7	4.7	5.0	5.1
834	200	4.0	3.7	3.7	3.8
	600	5.3	4.7	5.0	5.0
835	200	1.7	1.7	1.7	1.7
	600	5.7	4.7	5.0	5.1
836	200	3.7	3.7	3.0	3.5
	600	6.0	4.7	5.0	5.2
837	200	2.7	2.7	2.7	2.7
	600	5.0	5.0	5.3	5.1
838	200	2.0	2.7	2.7	2.5
	600	6.0	5.0	5.0	5.3
839	200	3.0	2.7	3.7	3.1
	600	5.0	5.0	5.3	5.1
842	200	3.3	4.3	4.2	3.9
	600	5.7	6.5	6.0	6.1
844	200	2.3	2.0	2.5	2.3
	600	4.7	4.2	4.0	4.3
853	200	2.7	2.7	2.7	2.7
	600	5.0	5.0	5.3	5.1
854	200	Not tested because of similarity to Sample No. 853			
	600				
Industrial grade xylene c/	200	5.0	3.7	4.0	4.2
	600	5.7	6.0	5.7	5.8

a/Each figure represents the mean of three weekly injury scale ratings.
b/Activity index number obtained by determining the average of the mean injury scale ratings for each species tested.
c/Used as a standard contact herbicidal solvent for comparative purposes.

Laboratory No. : 761

Compound : Potassium salt of 2-(2,4,5-trichlorophenoxy) propionic acid

Formulation : 22.8% Active Ingredient, 20% Acid Equivalent, Soil Applied Granular

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds									
Standing Water-Continuous Contact Test-Conducted in Greenhouse									
Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1 and 2 Weeks Following Treatment						Average Rating		
	Rate 5 #/A		Rate 20#/A		Rate 40#/A		5#/A	20#/A	40#/A
	1	2	1	2	1	2			
P. pectinatus	3	10	3	10	4	10	6.5	6.5	7
P. nodosus	2	10	2	10	2	10	6	6	6
E. canadensis	3	10	4	10	4	10	6.5	7	7

Laboratory No. : 762

Compound : Potassium salt of 2-(2,4,5-trichlorophenoxy) propionic acid

Formulation : 6# Acid Equivalent per Gallon, Soil Applied Liquid

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds									
Standing Water-Continuous Contact Test-Conducted in Greenhouse									
Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1 and 2 Weeks Following Treatment						Average Rating		
	Rate 5#/A		Rate 20#/A		Rate 40#/A		5#/A	20#/A	40#/A
	1	2	1	2	1	2			
P. pectinatus	1	10	2	10	4	10	5.5	6	7
P. nodosus	2	9	1	8	3	10	5.5	4.5	6.5
E. canadensis	2	10	2	10	2	10	6	6	6

Laboratory No. : 828

Compound : Crude methyl naphthalene

Formulation : Solvent, 100% Active Ingredient

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. ___ ppm				Herbicide Conc. ___ ppm				___ ppm	___ ppm
	1	2	3	4	1	2	3	4		
P. pectinatus										
P. nodosus										
E. canadensis										

Limited Contact Period Test For 30 Min. Period — Flowing Water ☐ — Standing Water ☒

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 200 ppm				Herbicide Conc. 600 ppm				200 ppm	600 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	2	1	0.5		3	3.5	1.5		1.2	2.7
P. nodosus	1	1	1		3	4	2		1	3
E. canadensis	1	1	1		5	5	3		1	4.3

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury; 4, 5, 6=Moderate Injury, 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Laboratory No. : 829

Compound : Refined methyl naphthalene

Formulation : Solvent, 100% Active Ingredient

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. ___ ppm				Herbicide Conc. ___ ppm				____ ppm	____ ppm
	1	2	3	4	1	2	3	4		
P. pectinatus										
P. nodosus										
E. canadensis										

Limited Contact Period Test For 30 Min. Period — Flowing Water ☐ — Standing Water ☒

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 200 ppm				Herbicide Conc. 600 ppm				200 ppm	600 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	3	4	3		3	4.5	4		3.3	3.8
P. nodosus	2	2	2		3	3	3		2	3
E. canadensis	1	1	1		3	3	2		1	2.7

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury, 4, 5, 6=Moderate Injury; 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Laboratory No. : 830

Compound : Tetrahydronaphthalene

Formulation : Solvent, 100% Active Ingredient

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. ___ ppm				Herbicide Conc. ___ ppm				____ ppm	____ ppm
	1	2	3	4	1	2	3	4		
P. pectinatus										
P. nodosus										
E. canadensis										

Limited Contact Period Test For 30 Min. Period — Flowing Water ☐ — Standing Water ☒

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 200 ppm				Herbicide Conc. 600 ppm				200 ppm	600 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	2	4	3		5	7	6.5		3	6.2
P. nodosus	2	2	3		5	6	6		2.3	5.7
E. canadensis	2	2	2		4	5	3		2	4

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury; 4, 5, 6=Moderate Injury; 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Laboratory No. : 848

Compound : 4-amino-3,5,6-trichloropicolinic acid

Formulation : 10% Active Ingredient, Clay Granules

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 5 ppm				Herbicide Conc. 100 ppm				5 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	0	0.5	0	2	7	9.5	10	10	0.6	7.8
P. nodosus	1	2	4	7.5	3	8	10	10	3.6	7.8
E. canadensis	3	4	4.5	8	7	9.5	10	10	4.9	9.1

Limited Contact Period Test For __ Min. Period Flowing Water ☐ Standing Water ☐

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. ___ ppm				Herbicide Conc. ___ ppm				___ ppm	___ ppm
	1	2	3	4	1	2	3	4		
P. pectinatus										
P. nodosus										
E. canadensis										

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury; 4, 5, 6=Moderate Injury; 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Laboratory No. : 849

Compound : 4-amino-3,5,6-trichloropicolinic acid

Formulation : 2 pounds per gallon Active Ingredient, Liquid

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 5 ppm				Herbicide Conc. 100 ppm				5 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	0	0	0	3	5	6	9	10	0.8	7.6
P. nodosus	1	1.5	2	3	3	5	9	10	1.9	6.8
E. canadensis	3	3	3	9	7	7	10	10	4.5	8.5

Limited Contact Period Test For ___ Min. Period Flowing Water ☐ Standing Water ☐

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. ___ ppm				Herbicide Conc. ___ ppm				___ ppm	___ ppm
	1	2	3	4	1	2	3	4		
P. pectinatus										
P. nodosus										
E. canadensis										

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury; 4, 5, 6=Moderate Injury; 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Laboratory No. : 855

Compound : Tris-(1-dodecyl-3-methyl-2-phenylbenzimidazolium) ferricyanide

Formulation : 25% Active Ingredient, Wettable Powder

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 5 ppm				Herbicide Conc. 100 ppm				5 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	8	10	10	10	8	10	10	10	9.5	9.5
P. nodosus	6	9	9	9	8.5	10	10	10	8.3	9.6
E. canadensis	8	9	9	9	10	10	10	10	8.7	10

Limited Contact Period Test For 30 Min. Period — Flowing Water ☐ — Standing Water ☒

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 5 ppm				Herbicide Conc. 100 ppm				5 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	0	0	0	0	5	4	3	3	0	3.8
P. nodosus	0	0	0	0	4	3	3	3	0	3
E. canadensis	0	0	0	0	6	6	4	3	0	4.8

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury; 4, 5, 6=Moderate Injury; 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Laboratory No. : 856

Compound : Bromine salt of tris-(1-dodecyl-3-methyl-2-phenylbenzimidazolium) ferricyanide

Formulation : 95% Active Ingredient, wettable powder

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 5 ppm				Herbicide Conc. 100 ppm				5 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	7	10	10	9.5	8	10	10	10	9.1	9.5
P. nodosus	6	9	10	10	8	10	10	10	8.5	9.5
E. canadensis	8	9	9	10	10	10	10	10	9	10

Limited Contact Period Test For 30 Min. Period — Flowing Water ☐ — Standing Water ☒

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 5 ppm				Herbicide Conc. 100 ppm				5 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	0	0	0	0	5	4	3	2	0	3.5
P. nodosus	0	0	0	0	4	4	3	2	0	3.3
E. canadensis	0	0	0	0	7	7	3	4	0	5.3

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury; 4, 5, 6=Moderate Injury; 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Laboratory No. : 825

Compound : Coco diamine

Formulation : 100% A.I. Liquid

Weekly injury scale ratings of compounds evaluated for herbicidal activity on aquatic weeds

Standing Water - Continuous Contact Test - Conducted in Greenhouse

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 50 ppm				Herbicide Conc. 100 ppm				50 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	10	10	10	10	10	10	10	10	10	10
P. nodosus	10	10	10	10	10	10	10	10	10	10
E. canadensis	10	10	10	10	10	10	10	10	10	10

Limited Contact Period Test For 30 Min. Period — Flowing Water [x] — Standing Water [ ]

Potted Cultures of Rooted Aquatic Weed Species	Injury Ratings 1, 2, 3, and 4 Weeks Following Treatment								Average Rating	
	Herbicide Conc. 5 ppm				Herbicide Conc. 100 ppm				5 ppm	100 ppm
	1	2	3	4	1	2	3	4		
P. pectinatus	0	0	0	1	7	6.5	7	6.5	0.3	6.8
P. nodosus	1	2	2	1.5	7	5.5	7	6.5	1.6	6.5
E. canadensis	4	5	5	4	7	6	6	5	4.50	6

Injury rating scale, 0=No injury; 1, 2, 3=Slight Injury; 4, 5, 6=Moderate Injury; 7, 8, 9=Severe Injury With Some Regrowth Inhibition Above Soil Line; 10=Complete Kill of Plants Above Soil Line

Results of these greenhouse evaluations of the compounds do not show any compound of exceeding promise for control of pondweeds in irrigation canals. The potassium salt of silvex (Nos. 761 and 762) did show good activity in soil applied tests, but generally these types of materials have not proved to be successful when applied in flowing water situations. Further development of specific application techniques and formulations for aquatic soil applied materials is required.

The naphthalene solvent series of tests were suggested by certain results obtained from field applications of crude aromatic solvents that contained naphthalene compounds. The tetrahydronaphthalene (No. 830) exhibited the most activity of the three. Some difficulty was encountered in emulsifying these solvents. Some exploratory work has been conducted using naphthalene compounds as additives to aromatic solvent to determine the possibilities of enhancing their herbicidal activity. Results of these tests are not yet complete.

A completely new herbicidal compound (Nos. 848 and 849) was made available this year and the compound structure only recently released. This material was found to be active on the pondweed species tested under continuous contact conditions. This material was not tested in a limited contact or flowing water situation because of its slow action in producing injury symptoms. Its possible usefulness in aquatic weed control in irrigation systems would be limited to static water or possibly soil application.

Compounds No. 855 and 856 exhibited good activity in static water tests, but with much reduced activity in flowing water limited contact tests. The potential usefulness of this compound in either formulations as an aquatic herbicide in irrigation canals is questionable.

Pondweed Propagule Production as Affected by Repeated Aromatic Solvent Treatments

Greenhouse experiments were conducted to study the effects of repeated aromatic solvent treatments on the vegetative propagule production of sago pondweed, Potamogeton pectinatus L., and American pondweed, P. nodosus Poir. These studies are being performed to determine the influence that continued aromatic solvent treatments may have on sago pondweed tuber and American pondweed winterbud production within a representative growing season. Data of this nature would be of potential use to field weed control personnel in determining the advantages or disadvantages of making multiple solvent treatments in addition to those required for normal vegetation control.

Tubers of sago pondweed and winterbuds of American pondweed were planted in 6-inch clay pots filled with topsoil. Each pot contained six propagules that were visually selected for uniformity in size and from the same propagule source during any treatment series. The potted propagules were cultured in either 1/2-cut 55-gallon steel barrels or full size 30-gallon steel barrels arranged in the greenhouse to receive uniform light and temperature conditions. Supplemental light from 150-watt incandescent flood lamps was used during short day periods.

A total of 18 pots of each species was planted for the first study series. This provided for a series of replicated pot treatments of six individuals for untreated checks, one treatment and two treatments each. Initial experimental layout called for four pot replications for a three-treatment series, but because of severe top kills obtained from a 300-ppm concentration of emulsified xylene only a two-treatment series was obtainable. Treatment chronology is tabulated in Table 10.

Treated cultures and untreated controls were randomly arranged in the culture aquaria to equalize any effects of excess herbicidal residues. All treated plants were thoroughly rinsed in running tapwater following treatment and returned to the culture aquaria for observation and further treatments.

Cultures were treated with industrial grade xylene emulsified with 2 percent (v/v) of an anionic-nonionic blend emulsifier. (Laboratory No. 755.) Treatment concentration in the first test series was 300 ppm for a contact period of 30 minutes. A herbicidal concentration of 250 ppm for a 30-minute contact period was used in the second test. The potted cultures were allowed to produce significant amounts of vegetative regrowth before being subjected to subsequent treatments. These periods varied between treatments and were dependent on the regrowth accrued between treatments and final harvest. Regrowth vigor varied between the treated cultures and with species. Potted cultures were selected for additional treatments on the basis of regrowth vigor.

Determinations were made of the number of vegetative propagules produced under a specified treatment and the respective fresh and dry weights of the propagules. Results of the first test series are tabulated in Tables 11 and 12. Results obtained from the second test series are itemized in Tables 13 and 14. Mean data contained in Tables 11, 12, 13, and 14 are graphically represented in Figure 1.

Data obtained in this study were analyzed for statistical significance by standard least square methods to determine means and standard error of means, as described by Schumacher and Chapman (15).

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Table 10

TREATMENT PROGRAM UTILIZED IN EVALUATING THE EFFECTS OF MULTIPLE SOLVENT TREATMENTS ON SAGO AND AMERICAN PONDWEED PROPAGULE PRODUCTION

Test series	Treatment	Species	Plant age in days at time of treatment	Solvent concentration (ppm)	Water temp during treatment, °F	General vegetative condition at time of treatment
1	1	Sago	48	300	73-76	Vigorous growth
1	1	American	37	300	70-75	Vigorous growth
1	2	Sago	153	300	68-70	Fair to poor regrowth
1	2	American	142	300	68-70	Good regrowth
1	Harvested	Sago	236	—	—	Fair regrowth--senescence
1	Harvested	American	225	—	—	Fair regrowth--senescence
2	1	Sago	44	250	60-65	Vigorous growth
2	1	American	44	250	60-65	Vigorous growth
2	2	Sago	89	250	60-65	Good regrowth
2	2	American	89	250	60-65	Good regrowth
2	3	Sago	252	250	65-70	Fair to poor regrowth
2	3	American	252	250	65-70	Fair to poor regrowth
2	Harvested	Sago	274	—	—	Fair to no regrowth-- some senescence
2	Harvested	American	274	—	—	Fair to poor regrowth-- some senescence

Table 11

EFFECTS OF AROMATIC SOLVENT TREATMENTS ON SAGO PONDWEED TUBER PRODUCTION, TEST SERIES NO. 1

Replication No. (pot) or sample	Treatment	No. of tubers produced/ sample	Total fresh weight of tubers/ sample, grams	Total oven dry weight of tubers/ sample, grams	Mean dry weight/ tuber, milligrams[a]	Percent dry weight of tubers
1	Check	41	4.395	1.592	39	36.22
2	Check	41	4.231	1.736	42	41.03
3	Check	40	4.371	1.774	44	40.58
4	Check	53	5.761	2.393	45	41.53
5	Check	41	5.409	2.167	53	40.06
6	Check	59	5.308	2.207	37	41.57
Means	--	45.83	--	--	[a] 43.33	[a] 40.32
1	1	38	4.040	1.546	41	38.26
2	1	16	2.265	0.798	50	35.23
3	1	26	2.284	0.832	32	36.42
4	1	48	3.628	1.182	25	32.57
5	1	15	2.048	0.667	44	32.56
6	1	35	2.867	0.881	25	30.72
Means	--	29.67	--	--	[a] 36.17	[a] 34.33
1	2	22	2.550	0.910	41	35.68
2	2	27	3.520	1.159	43	32.92
3	2	33	3.431	1.082	33	31.53
4	2	20	1.523	0.446	22	29.71
5	2	23	2.046	0.772	34	37.73
6	2	17	1.816	0.661	39	36.39
Means	--	23.67	--	--	[a] 35.33	[a] 34.83

[a]/Determined from rounded values.

Statistical analyses of the data in Table 11 indicate that sago pondweed tuber production was influenced by solvent treatment to the extent indicated by the following:

a. The mean number of tubers produced by the untreated plants was greater than one treatment (P* = 0.80) and two treatments (P = 0.95). One treatment tuber number was not significantly different from two treatments (P = 0.50, 1T > 2T).

b. A reduction in the average dry weight per tuber occurred between treated and untreated plants (P = 0.80 that check > 1T and P = 0.90 that check > 2T). Mean dry weights did not significantly differ between treatments.

*P = Probability.

Table 12

EFFECTS OF AROMATIC SOLVENT TREATMENTS ON AMERICAN PONDWEED WINTERBUD PRODUCTION, TEST SERIES 1

Replication No. (pot) or sample	Treatment	No. of buds produced/ sample	Total fresh weight of buds/ sample, grams	Total oven dry weight of buds/ sample, grams	Mean dry weight/ bud milligrams a/	Percent dry weight of buds
1	Check	74	14.363	5.159	70	36
2	Check	35	8.142	2.875	82	35
3	Check	49	10.155	3.620	74	36
4	Check	56	12.248	4.398	79	36
5	Check	62	12.744	4.381	71	34
6	Check	25	6.251	2.119	85	34
Means		50.17	-	—	a/ 76.83	a/ 35.17
1	1	19	[illegible].740	1.345	71	36
2	1	26	6.022	2.191	84	36
3	1	14	1.[illegible]74	0.458	33	31
4	1	27	4.[illegible]17	1.510	56	33
5	1	37	5.259	1.788	48	34
6	1	37	7.100	2.633	71	37
Means		26.67	—	—	a/ 60.50	a/ 34.50
1	2	18	3.703	1.180	66	32
2	2	30	4.384	1.405	47	32
3	2	33	6.002	2.014	61	34
4	2	34	6.588	2.266	67	34
5	2	17	3.787	1.233	73	33
6	2	32	5.745	2.019	63	35
Means		27.33	—	—	a/ 62.83	a/ 33.33

a/Determined from rounded values.

c. The average percent dry weight per tuber shows that untreated plants differed significantly from all treated plants (P = 0.99), but only slight variations occurred between one and two treatments.

Interpretation of data in Table 12 on production of American pondweed winterbuds is as follows.

a. Untreated plants produced a greater mean number of winterbuds than treated plants (P = 0.98). There were no significant differences in winterbud mean numbers between one and two treatments.

b. Average dry weight per winterbud was significantly greater with untreated plants as compared to treated plants ($P = 0.90$, checks 1T, and $P = 0.99$, checks > 2T). No significant differences occurred between the two treatments.

c. The average percent dry weight of winterbuds did not greatly differ between untreated plants and one treatment ($P = 0.30$, check > 1T), whereas untreated checks were significantly different from the second treatment ($P = 0.98$, check > 2T). There were some differences in mean percent dry weight of winterbuds between treatments ($P = 0.70$, 1T > 2T).

The results of the second test series that further evaluated the influence of solvent treatments on sago pondweed tuber production are tabulated in Table 13. Analyses of the results of this test, which include a third treatment in the series, are as follows:

a. Again the mean number of tubers produced by untreated plants exceeds that produced by those treated ($P = 0.99$). A greater mean number of tubers was produced under conditions of one treatment than under two treatments ($P = 0.95$, 1T > 2T). A considerable decrease in level of significance was exhibited between one treatment and three treatments ($P = 0.50$, 1T > 3T). Tuber number production was greater at the three-treatment level than two treatments.

b. Average dry weight of tubers produced by untreated plants was greater than all treated plants ($P = 0.99$). Mean tuber dry weights of one treatment were significantly greater than those treated twice ($P = 0.95$, 1T > 2T), but were less significant than those treated three times ($P = 0.50$, 1T > 3T). As with tuber number, average dry weight of three treatments was greater than two treatments.

c. The average percent dry weight per tuber indicates that untreated plants differ from treated plants ($P = 0.90$, check > 1T and 3T and $P = 0.50$, check > 2T). Mean percent dry weight of tubers differed significantly between treatments ($P = 0.99$, 1T > 2T and $P = 0.95$, 1T > 3T). Again the percent dry weight of three treatment tubers exceeded those under two treatments.

Table 13

EFFECTS OF AROMATIC SOLVENT ON SAGO PONDWEED TUBER PRODUCTION, TEST SERIES 2

Replication No. (pot) or sample	Treatment	No. of tubers produced/ sample	Total fresh weight of tubers/ sample, grams	Total oven dry weight of tubers/ samples, grams	Mean dry weight/ tuber, milligrams[a]	Percent dry weight of tubers
1	Check	124	13.7242	5.9364	48	39
2	Check	67	5.6674	2.0808	31	37
3	Check	68	10.9498	4.5517	67	42
4	Check	83	9.5903	3.9920	48	42
5	Check	67	8.6574	3.7046	56	43
6	Check	135	10.8721	4.3157	32	40
Means		90.66	9.9102	4.0969	a/ 47	a/ 40.5
1	1	39	3.4944	1.4711	38	42
2	1	63	5.1139	1.8590	30	36
3	1	52	4.8646	2.0210	39	42
4	1	51	2.3664	0.8837	17	37
Means		51.25	3.9598	1.5587	a/ 31	a/ 39.25
1	2	20	1.3208	0.3365	17	26
2	2	27	2.4305	0.5796	22	24
3	2	16	1.1973	0.3827	25	33
4	2	22	1.6232	0.3714	17	23
5	2	22	1.4987	0.4350	20	29
6	2	19	1.7800	0.5106	27	29
7	2	18	1.8812	0.4500	25	24
8	2	20	2.2396	0.5584	28	25
Means		20.50	1.7464	0.4530	a/ 22.62	a/ 26.63
1	3	32	2.8170	1.1130	35	40
2	3	25	3.1084	1.1200	45	36
3	3	52	3.1699	0.9610	19	30
4	3	16	0.8726	0.2724	17	31
Means		31.25	2.4919	0.8666	a/ 29.0	a/ 34.25

a/Determined from rounded values.

Table 14 lists the results of the second test series evaluating American pondweed winterbud production. These results are statistically interpreted as follows:

a. Average number of winterbuds produced by untreated plants was significantly greater than all treatments (P = 0.99). A greater mean number of winterbuds developed in one-treatment samples than in two- and three-treatment samples (P = 0.95, 1T > 2T and P = 0.99, 1T > 3T). Winterbud production was only slightly greater under conditions of two treatments than three treatments (P = 0.20, 2T > 3T).

b. Mean winterbud dry weights of untreated checks were significantly different than one and three treatments (P = 0.95, check > 1T and 3T), but was less than two-treatment cultures. One treatment mean dry weight production was significantly greater than three-treatment cultures (P = 0.99, 1T > 3T), but was less than two treatments. Two treatments produced winterbuds of greater dry weight than those under three treatments (P = 0.95).

c. An inverse relationship existed in the average percent dry weight of winterbuds, where all treatments had values greater than the untreated checks. Some differences occurred between percent dry weight of one treatment and two treatments (P = 0.50, 1T > 2T) and one treatment and three treatments (P = 0.90, 1T > 3T). Average percent dry weight of winterbud production under two treatments was significantly greater than three treatments (P = 0.90, 2T > 3T).

In general, the results of this laboratory study show that aromatic solvents have an influence on the vegetative propagule production of these two pondweed species under the conditions of this experiment. This is especially evident when comparing treated and untreated plants. Initial tests indicated that additional solvent treatments (beyond one treatment) did not significantly change the character of propagule production. The second test series illustrated that significant differences did occur between the one- and two-treatment series in both number of propagules and dry weight production for both species. The addition of a third treatment had no influence on reducing tuber and winterbud production, and in certain instances propagule production exceeded that of the two-treatment series.

Laboratory studies of this type present conditions that greatly limit the latitude of translating laboratory results to potential field application. In this experiment the relatively small size of experimental layout, both in size of plant clones and number of replications, would influence confidence in the final results. Another limitation to be considered is the herbicidal susceptibility of greenhouse grown plants,

Table 14

EFFECTS OF AROMATIC SOLVENT ON AMERICAN PONDWEED WINTERBUD PRODUCTION, TEST SERIES 2

Replication No. (pot) or sample	Treatment	No. of buds produced/ sample	Total fresh weight of buds/ sample, grams	Total oven dry weight of buds/ sample, grams	Mean dry weight/bud milligrams[a]	Percent dry weight of buds
1	Check	84	16.2283	5.2899	63	32
2	Check	86	12.8050	4.0566	47	32
3	Check	75	11.0515	3.4594	46	31
4	Check	59	11.3226	3.9291	67	35
5	Check	73	13.8754	4.4367	61	32
6	Check	60	12.4492	4.0746	68	38
Means		72.83	12.9553	4.2077	[a] 58.67	[a] 33.33
1	1	50	7.6156	2.8343	57	37
2	1	31	5.5569	1.9924	64	36
3	1	63	7.6778	2.8289	45	37
4	1	55	7.5675	2.8248	51	37
Means		49.75	7.1045	2.6201	[a] 54.25	[a] 36.75
1	2	30	5.3168	1.8424	61	35
2	2	33	4.5942	1.6354	50	36
3	2	44	7.2428	2.3349	53	32
4	2	27	5.3257	1.9994	74	38
Means		33.75	5.6199	1.9530	[a] 59.50	[a] 35.25
1	3	22	2.9601	1.0164	46	34
2	3	36	3.5302	1.0999	31	31
3	3	41	4.3868	1.5046	37	34
4	3	29	3.6720	1.1710	40	32
5	3	26	4.0476	1.3582	52	34
6	3	23	3.1738	1.0993	48	35
7	3	43	4.1570	1.2904	30	31
8	3	36	4.4103	1.3550	38	31
Means		32	3.7922	1.2369	[a] 40.25	[a] 32.75

[a]/Determined from rounded values.

that required considerable reduction in herbicidal concentration to allow for a maximum of only three treatments. The long periods required for vegetative regrowth between multiple treatments would also be expected to have some influence on vegetative propagule production. It was interesting to note that in all tests, regrowth apparently developed only from rhizome meristematic tissue and not from tubers or winterbuds which had never become vernalized.

The results of this study, while not conclusive, do show some important trends that could have practical field significance. The most interesting is that under the conditions of these experiments sago and American pondweed vegetative propagule production was significantly reduced by aromatic solvent treatment. Vegetative propagule production varied according to number of treatments in certain instances. A significant reduction in mean number and mean dry weight per tuber and winterbud was accrued between one treatment and two treatments, but no further reductions of tubers and winterbuds were evident with the third treatment. Further treatments beyond three were not possible because of limited amounts of vegetative regrowth.

Results obtained in these studies suggest that aromatic solvents in excess of two treatments will not further decrease the infestation potential of pondweed growth in the succeeding year, assuming most of the plant growth develops from tubers and winterbuds.

Additional investigations under conditions of flowing water in an outdoor situation were attempted during the past summer. Plant cultures did not develop sufficiently well for treatment and were discarded. The study was started late in the growing season and could not be repeated. Further investigation is anticipated.

Influence of Water Quality on the Herbicidal Effectiveness of Acrolein

The aquatic herbicide acrolein (Laboratory No. 816) was tested for effectiveness on submersed aquatic weeds in waters of varying alkalinity and high total dissolved solids. Information obtained from these studies would be of interest to field personnel in evaluating the potential usefulness of acrolein as an aquatic herbicide in situations where water quality is highly variable.

Synthetic alkaline waters, utilized in herbicidal evaluation tests, were made with sodium bicarbonate. Concentrations of the bicarbonate ion (HCO_3^{-1}) were determined by standard titration methods (16).

Synthetic hard waters used in herbicidal evaluation tests were made by the addition of various salts to tapwater according to recommendations made by the Chemistry Section of the Research Division. The chemical

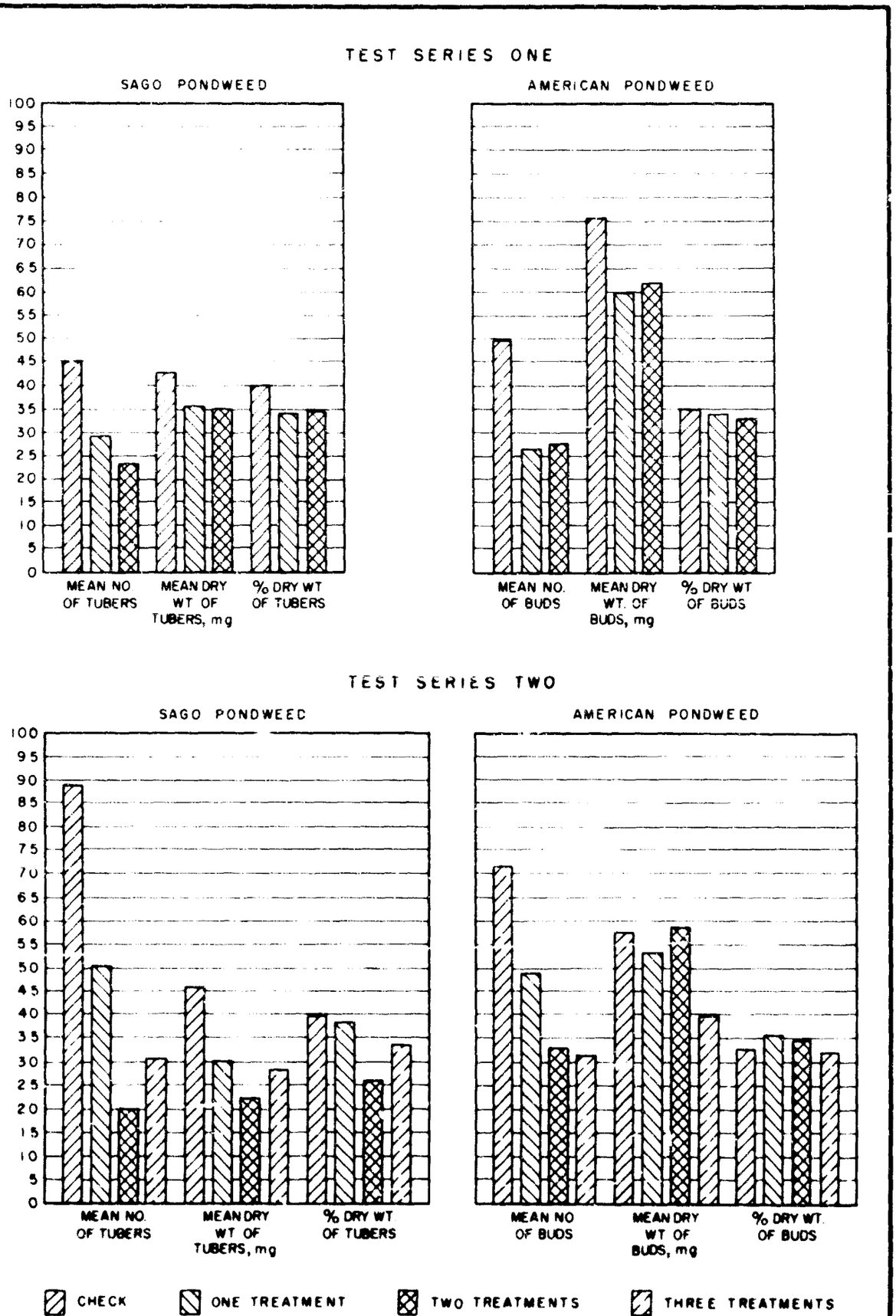

FIGURE 1 - PONDWEED PROPAGULE PRODUCTION AS AFFECTED BY AROMATIC SOLVENT TREATMENT

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components of these synthetic waters and laboratory tapwater are listed in Table 15. Tapwater was used as a comparative standard in all herbicidal tests evaluating water quality effect on plant response.

Table 15

ANALYSES OF WATER USED IN HERBICIDAL EVALUATION TESTS OF ACROLEIN

Laboratory tapwater	
TDS	78.0 ppm
Ca	13.0 ppm
Mg	2.3 ppm
Na	2.1 ppm
K	0.8 ppm
CO_3	0.0 ppm
HCO_3	28.0 ppm
SO_4	21.0 ppm
Cl	0.7 ppm
Total hardness expressed as $CaCO_3$	42.0 ppm

Synthetic hard water No. 1	
TDS	668 ppm
Ca	89.2 ppm
Mg	20.9 ppm
Na	59.6 ppm
K	3.7 ppm
HCO_3	34.4 ppm
SO_4	247.6 ppm
Cl	35.5 ppm
Total hardness expressed as $CaCO_3$	308.5 ppm

Synthetic hard water No. 2	
TDS	1,115.8 ppm
Ca	165.4 ppm
Mg	39.3 ppm
Na	117.1 ppm
K	6.7 ppm
HCO_3	40.8 ppm
SO_4	676.2 ppm
Cl	70.3 ppm
Total hardness expressed as $CaCO_3$	575.0 ppm

Pondweed species used in the standing water herbicidal evaluation test were potted cultures of sago pondweed, Potamogeton pectinatus and American pondweed, P. nodosus. All plants were cultured and held for observation in tapwater. Treatment conditions consisted of treating plants with three herbicidal concentrations and contact periods in waters varying in both alkalinity and total hardness.

Plants were held for a period of 2 weeks and injury ratings obtained weekly. Injury rating system is based on a 0 to 10 scale as previously described.

Water temperatures during herbicidal treatment and rinsing periods were maintained in a range of 67° to 70° F.

The results of these studies are tabulated according to individual tests and are reported as follows:

Table 16

EFFECTS OF HIGHLY ALKALINE WATERS ON THE HERBICIDAL ACTIVITY OF TWO PONDWEED SPECIES TREATED WITH ACROLEIN

Herbicidal treatment	Alkalinity of water, bicarbonate anion in ppm	Injury scale rating, mean of 2 weekly ratings: P. pectinatus	P. nodosus	Overall injury rating of both species
Acrolein 10 ppm 6-hour contact period	28	6.8	5.5	6.2
	84	6.5	6.0	6.3
	284	7.5	6.3	6.9
Acrolein 25 ppm 3-hour contact period	28	7.5	7.0	7.3
	84	7.5	6.5	7.0
	284	7.5	7.5	7.0
Acrolein 150 ppm 30-minute contact period	28	7.0	6.8	6.9
	84	6.3	7.0	6.7
	284	7.5	7.0	7.3
Untreated check*	84	0	0	0
Untreated check	284	0	0	0

*Plants held in highly alkaline waters for 2 weeks during injury observation.

The results of the test on herbicidal effectiveness of acrolein in highly alkaline waters, shown in Table 16, indicate no significant difference in injury between treatments. There was a slight decline in overall herbicidal activity at the 10-ppm, 6-hour contact period test. This reduced activity at lower concentrations of acrolein has been previously noted in past tests with this herbicide.

Table 17

EFFECTS OF INCREASED WATER HARDNESS ON THE HERBICIDAL ACTIVITY OF TWO PONDWEED SPECIES TREATED WITH ACROLEIN

Herbicidal treatment	Total hardness of treatment water in ppm, expressed as $CaCO_3$		Injury scale rating, mean of 2 weekly ratings: P. pectinatus	P. nodosus	Overall injury rating of both species
Acrolein 10 ppm 6-hour contact period	42	Tapwater	3.0	4.3	3.7
	308.5	Water No. 1	3.5	4.3	3.9
	575.0	Water No. 2	5.0	4.3	4.7
Acrolein 25 ppm 3-hour contact period	42	Tapwater	6.8	6.8	6.8
	308.5	Water No. 1	6.5	7.0	6.8
	575.0	Water No. 2	6.8	7.0	6.9
Acrolein 150 ppm 30-minute contact period	42	Tapwater	6.3	6.5	6.4
	308.5	Water No. 1	6.3	6.8	6.6
	575.0	Water No. 2	6.5	7.0	6.8
Untreated check*	308.5	Water No. 1	0	0	0
Untreated check	575.0	Water No. 2	0	0	0

*Plants held in waters of high total ($CaCO_3$) hardness for 2 weeks during injury observation.

The results of tests on herbicidal effectiveness of acrolein in hard waters, shown in Table 17, indicate that water hardness had no significant influence on resulting plant injury. As with the alkalinity test there was a significant decline in overall injury at the 10-ppm, 6-hour contact period test. This difference was considerably greater in the latter

test. Both injury ratings should be similar in the tapwater treatment. The only explanation that can be offered for these differences was the age of the plants in the separate tests. Plants used in the water hardness test were 24 days old at treatment while those used in the alkalinity test were 29 days old. Previous studies with aromatic solvents indicate significant reductions in injury with younger plants.

The results of these tests are not conclusive due to limitations inherent in laboratory tests, but indicate that the herbicidal activity of acrolein is not greatly influenced by changes in water alkalinity or hardness. A consideration that would have to be made in these tests is that the plants were not cultured in the waters of varying quality. It is possible that this might have some effect on subsequent herbicidal response, although various other physical factors are expected to have more influence on the herbicidal response of plants to acrolein than water quality. These conditions might best be met in a field situation.

Comparison of Pondweed Herbicidal Response to Aromatic Solvents in Flowing Versus Standing Water

Laboratory evaluation of the herbicidal activity of aromatic solvents has always been conducted in a flowing water situation rather than in static water. The flowing water situation was thought to present a more rigorous situation for evaluating a candidate solvent. This hypothesis was based mainly on empirical observation and not controlled experimental conditions. In these tests plants were always cultured under static conditions, treated in flowing water and held for observation in standing water. Prior to the fabrication of a larger model canal system where outside ponded water can be recirculated through a flume, a systematic evaluation of the various ramifications of flowing versus standing water effects on herbicidal response could not be evaluated.

The model canal system used for these preliminary studies was fabricated in the laboratories shop and installed in one of the greenhouses, as shown in Figure 2.

A small pond outside of the greenhouse was lined with butyl rubber and filled with tapwater, Figure 3. This water source is used as reservoir for recirculating water through the model test flume.

The outdoor pond water can be recirculated through the test flume at a maximum volume of about 1/3 cfs. Water temperature was monitored throughout the study and ranged from 63-70° F. The canal flume is constructed so as to provide a few inches of water over the plants in case of pump failure, which has been experienced on numerous occasions since original construction. A number of modifications were made in the system prior to a final reliable period of operation during the 1963 summer season.

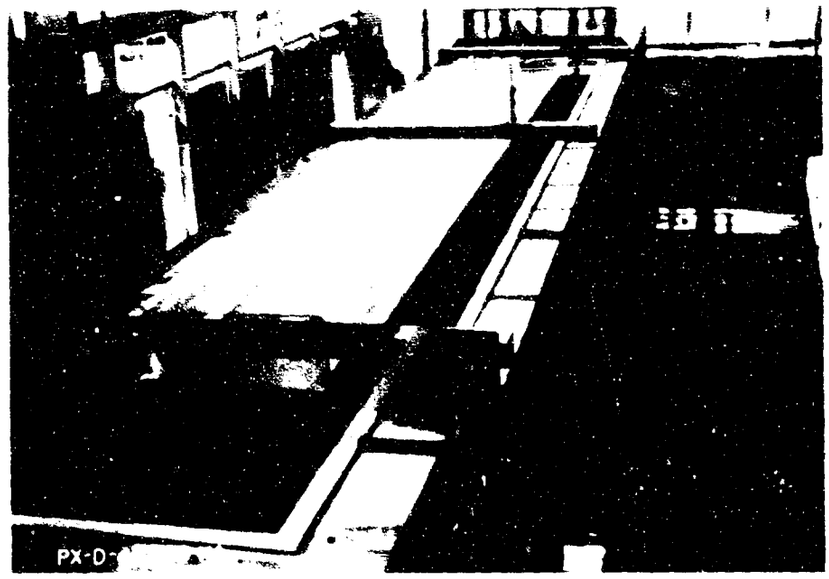

Figure 2. Model canal system utilized to recirculate water from a small outdoor pond for detailed study of pondweeds in a flowing water situation.

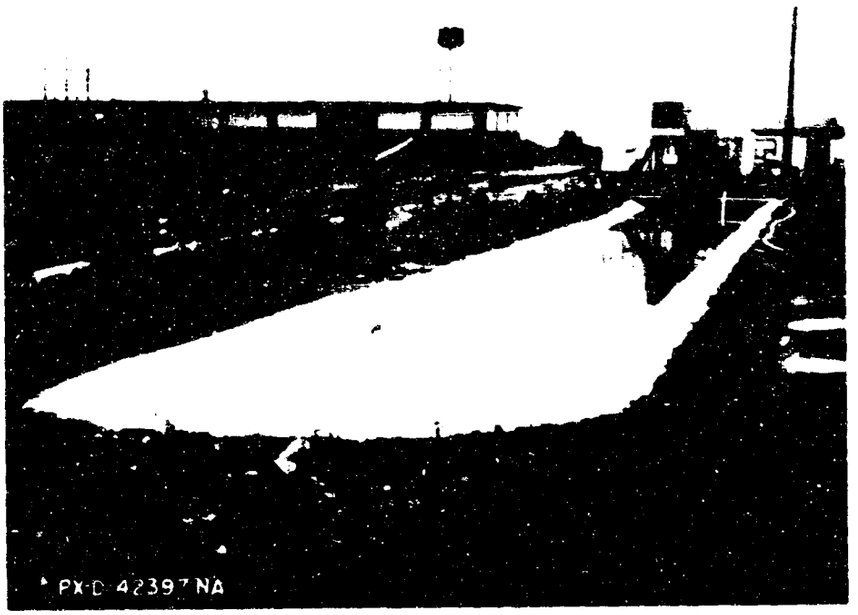

Figure 3. Small outdoor pond used as a water supply reservoir for recirculating water through the model test flume.

This preliminary study was designed to evaluate a multiple comparison of the effects of flowing and standing water on plant growth and herbicidal response to aromatic solvent herbicides. The various comparisons made are listed as follows:

Standing Water Culture Plants grown in 20-liter aquaria	Flowing Water Culture Plants grown in recirculated water of model canal
1A-Flowing water treatment-- 60-minute standing water rinse	1B-Flowing water treatment-- 60-minute standing water rinse
2A-Flowing water treatment-- 60-minute flowing water rinse	2B-Flowing water treatment-- 60-minute flowing water rinse
3A-Standing water treatment 60-minute flowing water rinse	3B-Standing water treatment-- 60-minute flowing water rinse
4A-Standing water treatment-- 60-minute standing water rinse	4B-Standing water treatment-- 60-minute standing water rinse

All flowing water rinses were made in the test flume that is equipped with three separated channels, any one of which can be set to waste the water carried in it. All rinse water was wasted to prevent contamination of the reservoir water. Standing water rinses were made in 20-liter plastic aquaria. Flowing water treatments were made in the smaller recirculating test flume used in evaluating aromatic solvent herbicides. This equipment is described in the aromatic solvent evaluation section of this report. Plants removed from any culture situation for treatment and rinsing were returned to the same flowing or static water condition for the 4-week period of injury observation.

A herbicidal concentration of 300 ppm of xylene, emulsified with 1-1/2 percent v/v of a nonionic-anionic emulsifier (Laboratory No. 755) was used in making all pondweed treatments. Water temperature during treatment and rinse periods was maintained from 65-70° F.

The two pondweed species included in this study were potted cultures of sago and American pondweed. Soil-filled 4-inch clay pots were utilized to facilitate movement of the cultures through the situations described. Replicated cultures of each species were tested under the conditions indicated. The overall test was not replicated at this time because of the late season start of the experiment. This was due primarily to mechanical problems associated with the equipment during the early summer season.

Observational injury ratings are the same as those used in evaluating solvents for herbicidal effect, as previously described. A total of four weekly injury ratings were made following treatment. Weekly injury ratings for each species-treatment condition are the means of two replicated pots. Only the first week injury rating on flowing water plants was made in the flowing situation because of pump motor flooding. The remaining three ratings were made with plants held in static water.

A number of untreated check plants of each species were grown under the flowing and static water conditions for observations and measurement of growth characteristics. Some slight differences were noted among cultures. Mean measurements typical of plants grown in each environment are summarized in Table 18.

Resulting weekly injury ratings for each species and treatment-rinse condition were plotted on coordinated paper and visual estimates of comparative relationships made. It was obvious that little differences occurred between conditions of standing water versus flowing water rinse, regardless of culture, species or culture condition, as seen in Figures 4 and 5. The two rinsing component data were combined for statistical comparisons of remaining culture and treatment differences that might occur. These data were subjected to linear regression analysis and

individual regressions plotted (Figures 4 and 5) according to culture conditions. Test for nonlinear components were not made at this time because of the preliminary nature of the study. Analyses of variance were computed between regressions of species, treatment condition, and culture conditions and significance both to means and slope were made. The significance of resulting comparison of injury obtained under the specified condition is as follows:

Figure 4 (Standing Water Culture) illustrates the significance of differences between species with respect to flowing or standing water treatment, American pondweed showing a significance of only P = 0.30 of mean difference and P = 0.50 of slope difference between two water treatment conditions. On the other hand, sago pondweed exhibited a significant effect of water movement during treatment with the analysis showing the significance of P = 0.90 that means differed and P = 0.80 that slopes differed. Mean injuries differed significantly when comparing sago with American in flowing water treatments, P = 0.70 that means differed, but only P = 0.20 that slopes differed. Likewise, when making the two species comparisons in standing water cultures the means differed by a P = 0.99, but with little significance in slope, P = 0.40.

A considerable reduction in overall injury obtained by treatments when plants were grown under flowing water conditions is illustrated in Figure 5, except for American pondweed treated in standing water. It should be noted that many American pondweed plants developed rather atypical during the study and results would be questionable. Analytical comparisons between the two culture conditions were not made because of the obvious significant differences. Plants grown in the flowing water were quite heavily encrusted with what appeared to be a mixture of calcareous material and epiphytic microorganisms. This would undoubtedly influence the rate or amount of absorption of the herbicidal solvent and subsequently reduce injury.

A breakdown of the significance between flowing water and static water treatment injury for sago pondweed exhibited significant difference of mean P = 0.90 and slope, P = 0.98. American pondweed also exhibited significant differences between standing and flowing water treatment with means (P = 0.99) that slopes differed less with P = 0.60. Comparisons between species under each treatment conditions indicate little significance in flowing water treatments, means P = 0.20 and slope P = 0.60 while under standing water conditions of treatment a significant mean difference occurred, P = 0.98, slope 0.60.

In summarizing it appears that under the conditions of this preliminary study, plants grown in flowing water exhibited a reduction in injury to aromatic solvent treatments as opposed to standing water cultures. Both sago pondweed and American pondweed showed some reduced injury

Table 18

GROWTH MEASUREMENTS OF SAGO AND AMERICAN PONDWEEDS GROWING UNDER CONDITIONS OF FLOWING AND STANDING WATER

Species and plant age	Means of growth measurements obtained on 2-week- and 6-week-old plants							
	Flowing water culture				Standing water culture			
	Terminal length, cm	Dry weight, grams	No. of branches	No. of leaves	Terminal length, cm	Dry weight, grams	No. of branches	No. of leaves
2-week plants								
Sago pondweed	18.5	0.7337	5.8	--	21.5	0.6178	4.8	--
American pondweed	31.5	0.376	--	4-Submersed 0-Floating	37.5	0.2884	--	4-Submersed 0-Floating
6-week plants								
Sago pondweed	33.0	1.3227	13.5	--	52.0	1.7304	11.5	--
American pondweed	69.0	0.7065	--	8.3 Submersed 3.6-Floating	58.5	1.4516	--	7.5-Submersed 4.5-Floating

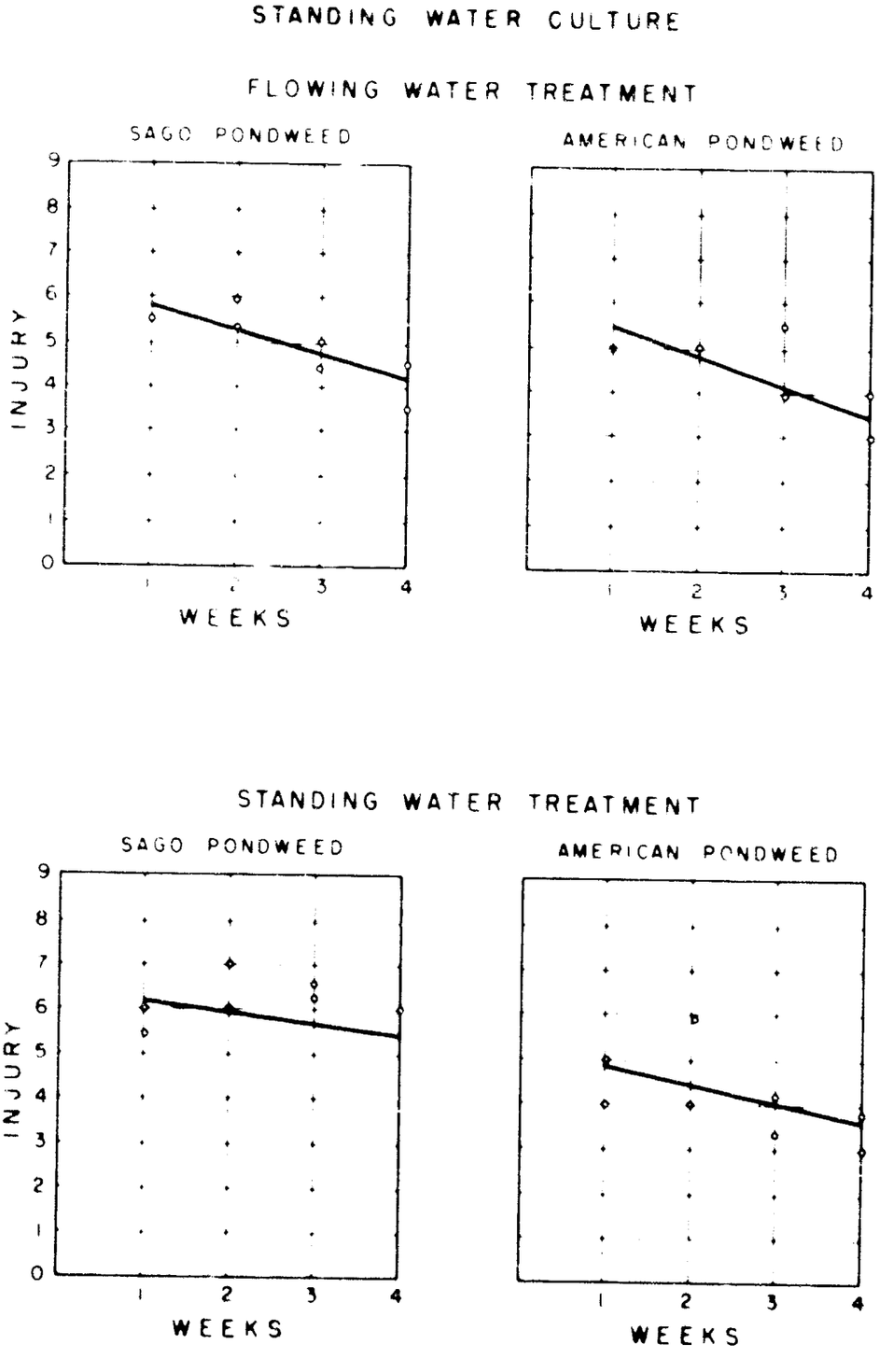

FIGURE 4 – INJURY OBTAINED ON TWO PONDWEED SPECIES GROWN IN STANDING WATER AND TREATED WITH EMULSIFIED XYLENE IN FLOWING OR STANDING WATER. REGRESSIONS OF INJURY ON WEEKLY RATING PERIODS ARE INDICATED BY SOLID LINES.

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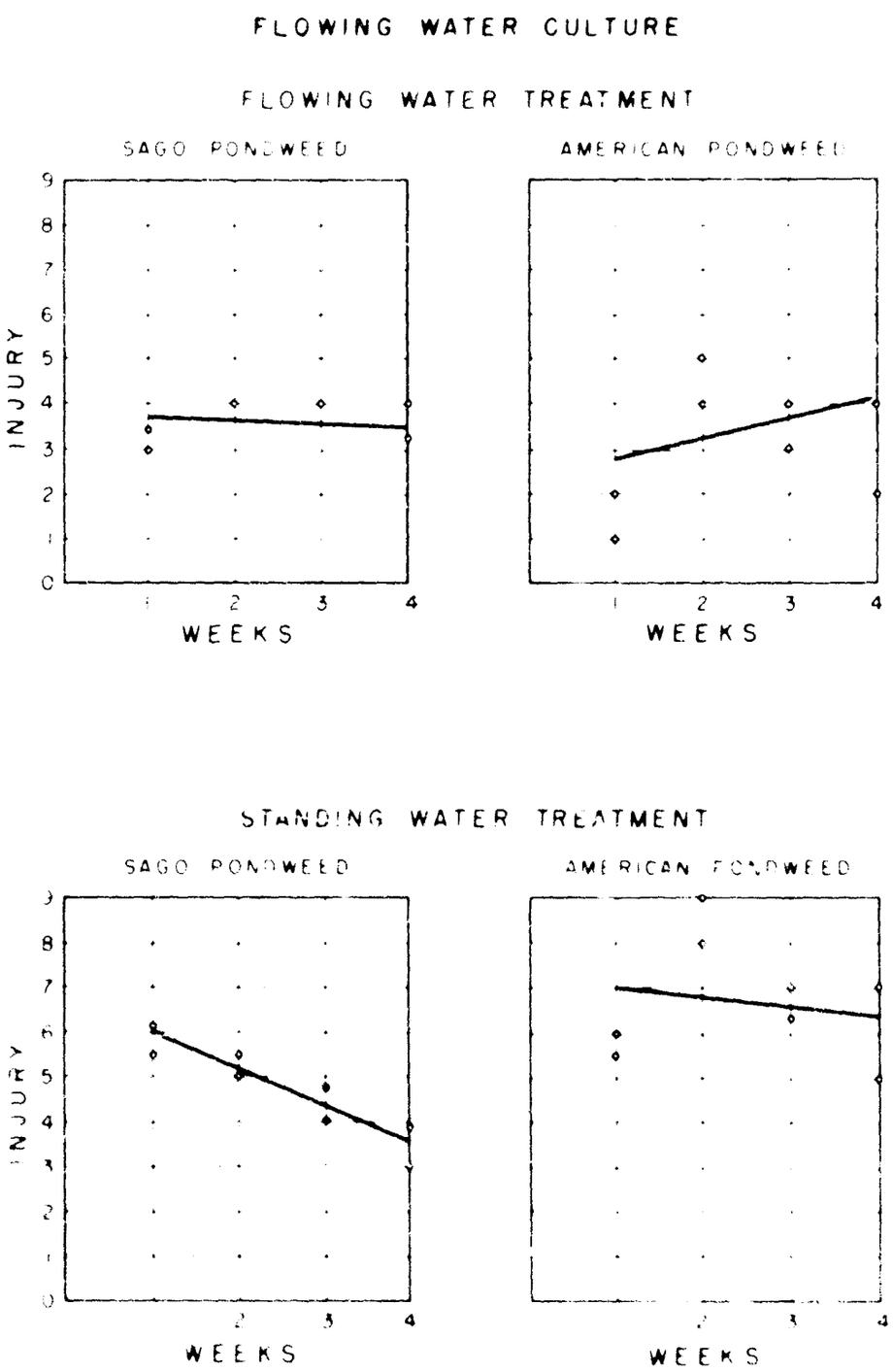

FIGURE 5 - INJURY OBTAINED ON TWO PONDWEED SPECIES GROWN IN FLC ING WATER AND TREATED WITH EMULSIFIED XYLENE IN FLOWING OR STANDING WATER REGRESSIONS OF INJURY ON WEEKLY RATING PERIODS ARE INDICATED BY SOLID LINES.

when treated in flowing water as opposed to standing water when cultured under either water condition. From the preliminary results, it appears that treatment of pondweeds under flowing water conditions might be justified in the laboratory evaluation of aromatic solvent herbicides. Also, it would appear desirable to culture plants under flowing water conditions for these tests, but with the present equipment and facilities it is impractical. Effects of rinsing regardless of flowing water or standing water do not apparently affect the overall obtainable injury. Previous unreported laboratory tests show that adequate rinsing period must follow a solvent treatment to obtain reliable injury results.

Effects of Water Temperature and Hardness on Emulsion Stability

Certain aromatic hydrocarbon solvents (13) are used to control submersed aquatic weeds in irrigation waters having a wide range of hardness and temperature. The emulsifying agent used in dispersing aromatic solvents is therefore expected to perform satisfactorily under a wide variation in each of these two factors.

This study was conducted to determine the influence of water temperature and hardness on emulsion stability. Water temperatures of 50, 60, 70 and 80° F were selected to represent the water temperature range expected in most irrigation waters. Waters containing 42 and 342 ppm hardness expressed as calcium carbonate were used for low and high hardness waters. For simplicity reasons, the low and high hardness waters will be referred to hereafter as soft and hard waters, respectively. Denver tapwater was used for the 42 ppm hardness water while the 342 ppm hard water was made by adding calcium chloride and magnesium chloride to distilled water.

The four emulsifiers used to evaluate the two factors represent both nonionic and nonionic-anionic blend materials. Numbers were assigned to the agents for identification purposes. Each of the materials was dissolved in xylene to give emulsifier concentrations of 1.0, 1.5 and 2.0 percent w/v in the emulsifiable concentrate. Each of these materials has been evaluated in hard water previously and reported to Bureau offices for their information. Therefore, the results for hard water at room temperature were anticipated.

The laboratory test method described in Chemical Engineering Laboratory Report No. W-1 (12) was used in preparing the emulsions and determining the resulting amount of cream layer and free oil. Temperatures were maintained by keeping the water and concentrates in a constant temperature water bath prior to preparing the emulsions and the prepared emulsions in the bath during the 4 hours while the readings on amount of cream and oil were made.

Tests were run in triplicate on each emulsifier used at three different concentrations in both soft and hard water and at four different water temperatures. The average amount of cream and oil for each replicated test is given in Table 19.

These data show that there is considerable difference in emulsion stability among the four emulsifiers, and the emulsion stability generally decreases with a decrease in emulsifier concentration. The hardness of the water also influences the stability of the emulsions.

Amount of cream layer formed during the 4-hour period is considered the most important of the three reading periods. In Figure 6 the average for each 4-hour reading is plotted to show a comparison of emulsion stability among emulsifiers, emulsifier concentration, water temperature and water hardness.

From the data presented, it can be concluded that the emulsifiers produce greater emulsion stability in the hard water, and in most cases water temperature does not affect emulsion stability to any significant degree. Emulsifier No. 2 in hard water at the 1.5-percent level is an exception in that it showed a sharp decline in stability in 50° water. Emulsifier No. 3 used at the 2-percent level is the only material that produced good emulsion stability in both soft and hard water and at all water temperatures.

In selecting emulsifying agents used in this study for best performance under certain conditions of water temperature and hardness, the test results indicate the following:

1. Emulsifier No. 3 at 2 percent for a wide variation in water hardness and temperature.

2. Emulsifier No. 2 at 1.5 percent for hard waters of the higher temperatures.

3. Emulsifier No. 1 or No. 3 at 1.5 percent for hard waters of the lower temperatures.

Pelletized Aquatic Herbicides

Herbicides are not normally formulated specifically for application to aquatic soils in flowing water situations. These materials possess certain physical characteristics such as rate of dissolution and degradation that are not optimum for extended effectiveness when used in treating aquatic soils of irrigation canals.

Table 19

RESULTS OF EMULSION STABILITY TEST

Average Divisions of Cream (cr) and Oil (o) on Triplicate Tests After Standing for 1, 2, and 4 Hours for Four Emulsifiers Used at 1.0, 1.5, and 2.0 Percent Levels in Four Water Temperatures and in Both Soft and Hard Water

Laboratory No.	Emulsifier No.	Percent emulsifier	Water hardness (ppm $CaCO_3$)	80°						70°						60°						50°					
				1 hr		2 hr		4 hr		1 hr		2 hr		4 hr		1 hr		2 hr		4 hr		1 hr		2 hr		4 hr	
				Cr	o	Cr	o	Cr	o	Cr	o	Cr	o	Cr	o	Cr	o	Cr	o	Cr	o	Cr	o	Cr	o	Cr	o
755	1	1	342	14	0	23	0	33	t	11	0	20	0	31	0	2	0	5	0	12	0	3	0	6	0	13	0
		1.5		3	0	7	0	14	t	3	0	5	0	14	t	1	0	2	0	3	0	0	0	2	0	4	0
		2.0		1	0	1	0	3	1	t	0	3	0	4	0	0	0	2	0	2	0	0	0	1	0	2	0
	1	1	42	32	0	33	0	33	0	30	0	33	0	33	0	28	0	32	0	32	0	29	0	32	0	32	0
		1.5		13	0	27	0	34	0	25	0	30	0	34	0	9	0	18	0	29	0	17	0	26	0	32	0
		2.0		4	0	8	0	16	0	3	0	10	0	23	0	2	0	4	0	9	0	7	0	16	0	25	0
748	2	1	342	8	0	17	T	25	t	32	0	30	3	27	4	23	0	29	0	31	1	31	0	34	0	34	t
		1.5		3	0	3	T	3	t	2	0	3	0	8	0	3	0	6	0	11	0	24	0	33	0	37	0
		2.0		t	0	1	0	1	t	0	0	2	0	2	0	0	0	2	0	2	0	2	0	5	0	10	0
	2	1	42	0	0	28	0	39	0	34	0	35	0	35	t	31	0	32	0	31	0	31	0	31	0	31	0
		1.5		12	0	3	0	39	0	27	0	39	0	43	0	28	0	34	0	39	0	30	0	34	0	36	0
		2.0		6	0	1	0	24	0	11	0	21	0	33	0	11	0	22	0	34	0	23	0	29	0	29	0
535	3	1	342	14	0	22	0	32	0	8	0	17	0	28	0	8	0	17	0	28	0	10	0	16	1	28	1
		1.5		2	0	6	0	12	0	2	0	4	0	9	0	1	0	4	0	10	0	1	0	3	0	8	0
		2.0		1	0	3	0	5	t	1	0	2	0	4	0	1	0	2	0	4	0	t	0	1	0	2	0
	3	1	42	26	0	36	0	38	0	26	0	35	0	38	0	34	0	38	0	37	0	34	0	36	0	34	0
		1.5		6	0	14	0	26	0	4	0	12	0	23	0	7	0	17	0	29	0	7	0	15	0	27	0
		2.0		2	0	4	0	10	0	1	0	4	0	7	0	1	0	2	0	6	0	1	0	4	0	7	0
577	4	1	342	16	0	26	t	28	1	32	0	36	0	37	0	26	0	31	0	32	0	30	t	31	t	30	1
		1.5		22	T	30	t	33	1	20	0	30	0	35	0	16	0	23	0	31	0	19	0	24	0	29	0
		2.0		8	0	15	t	24	t	10	0	20	0	27	0	7	0	14	0	21	0	9	0	15	0	21	0
	4	1	42	29	0	38	t	40	t	27	0	37	0	38	0	32	0	38	0	40	0	19	0	23	0	30	0
		1.5		16	0	28	t	40	t	21	0	23	0	41	t	19	0	33	0	40	0	21	0	31	0	39	0
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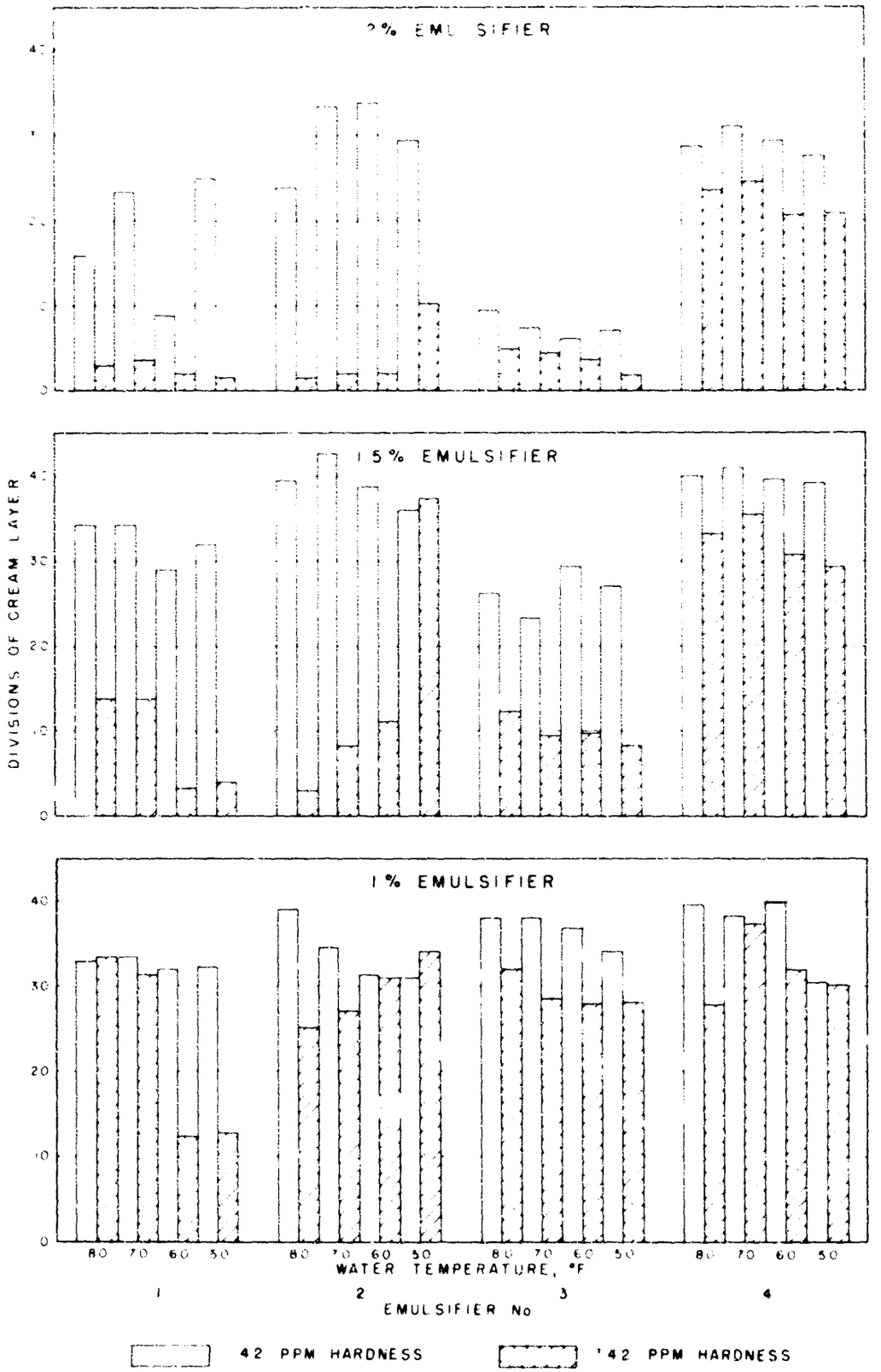

FIGURE 6 – THE AMOUNT OF CREAM REPRESENTS THE AVERAGE OF TRIPLICATE TESTS OVER 4 HOURS FOR 4 EMULSIFIERS TESTED AT 3 PERCENTAGES IN BOTH SOFT AND HARD WATER

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It is the intent of this study to investigate the potentials of incorporating some of the more active aquatic herbicides into a resinous binder to prepare an aquatic herbicide formulation having characteristics more compatible with some of the requirements believed necessary for good performance under field conditions. The principle of controlled release of a toxicant has worked very well in extending the activity of algaecides included in antifouling paints for inhibiting the attachment of algae to the surfaces of irrigation structures. Limited work performed on pelletized herbicide formulations has been made in an attempt to duplicate the formulation principle used in fouling prevention materials.

The resinous binder used in preparing the herbicide pellets is a vinyl resin copolymer solution, vinyl chloride-vinyl acetate dissolved in solvents, and is referred to in Bureau paint specifications as VR-6 seal coat (17). Pellets are prepared by mixing the herbicide into the vinyl resin solution until the mixture reaches a thick paste consistency. The paste is transferred to a hypodermic syringe and extruded in a continuous small diameter extrusion onto a glass surface for drying. The air-dried formulation is broken into small pieces which are used for soil applications. Percentage of active ingredient (herbicide) in the pellets is computed from the weight of the ingredients.

The pelletized herbicides are evaluated for activity by treating soil contained in 6-inch flower pots which is planted with sago pondweed tubers. A test in progress showing sago pondweed growth from treated and untreated soil is illustrated in Figure 7.

The pelletized material is applied to the top 1 inch of soil on a pound-per-acre rate based on active ingredient. Providing the herbicide treatment gives a complete kill of the initial crop of pondweed, the water is drained from the aquaria and the soil replanted with tubers. Fresh tapwater is used to refill the aquaria. This procedure is repeated following each complete kill.

Five different pelletized herbicide formulations were included in the first series of tests to determine their activity in a resinous binder. The herbicides used were 2,4-D acid, monuron, fenac amide, 2,4-D acid plus sand and monuron plus 2,4-D acid. The results of this test are given in Table 20.

All formulations gave complete kill of at least one crop at all rates tested. Monuron and fenac produced two complete kills at the 40-pound rate and above. The 2,4-D acid did not show the longevity of activity at the 40-pound rate as did these two compounds.

Figure 7. A comparison of sago pondweed growth from untreated soil on the left and from soil treated with herbicide pellets on the right.

Table 20

EFFECTS OF PELLETIZED HERBICIDES ON SAGO PONDWEED BY NUMBER OF CROPS ON WHICH COMPLETE KILL WAS OBTAINED

Herbicide	Rates of application (pounds per acre a.i.)			
	10	20	40	60
2,4-D acid	1	1	1	--
Monuron	1	1	2	2
Fenac amide	1	1	2	--
2,4-D acid plus sand	1	1	1	--
Monuron and 2,4-D acid*	1	--	--	--

*Contains 56.1 percent monuron and 43.9 percent 2,4-D on a.i. basis.

In the second series of tests, the activity of pelletized monuron was compared with that of the wettable powder form of monuron by the same test procedure. Duplicate tests were run on 10-, 20-, 40- and 80-pound-

per-acre rates for both the pellets and wettable powder. Test results are given in Table 21.

Table 21

TOTAL NUMBER OF CROPS OF SAGO PONDWEED ERADICATED BY MONURON IN THE FORMS OF WETTABLE POWDER AND PELLET

Monuron formulation	Test No.	Rates of application (pounds per acre a.i.) 10	20	40	80
Wettable	1	1	2	3	3
Powder	2	1	1	2	4
Pellet	1	1	2	3	3
	2	1	[illegible]	3	4

The resinous binder did not extend the period of activity of the compound at the 10-pound rate. However, at the 20- and 40-pound rates, the pelletized material showed a slight increase in total number of crops killed over that of the wettable powder. Although this would indicate that the resin has increased the total activity of certain treatments, the data are considered too limited for any definite conclusions.

Additional studies are continuing to determine the potential of using this type resin for preparing pelletized materials of longer herbicidal activity for controlling submersed aquatic weeds on irrigation systems. The ratio of herbicide to the resin may be an important factor in the controlled release of the phytotoxicant that will be given consideration.

FIELD STUDIES

Preliminary Survey of Interstitial Oxygen in Aquatic Soils

Field studies of the submersed aquatic environment have in certain instances suggested a possible relationship between aquatic soil texture and the degree of pondweed infestations. There have been some indications that certain canal soils containing high clay and fine silt fractions are less densely infested or completely lack rooted pondweed growths. This may be simply due to lack of the plants ability to penetrate the more dense substrate and become established. Although little is known about the soil-oxygen requirements for pondweed root zones, it is known to be a necessary requirement for normal development of the roots of terrestrial plants. In the case of rooted aquatic plants, the oxygen source would be in a dissolved state. Interstitial soil oxygen has not been previously included in our studies because of

the lack of a suitable sampling and analytical technique. A recent publication described a method to rapidly obtain water samples from the interstitial spaces of aquatic soils in undisturbed natural sites (18). This technique was explored during the past summer in the laboratory and at various field sites and found to provide reliable data on soil-water oxygen levels.

The equipment utilized and detailed description of sampling and chemical analytical procedures are described in the appendix of this report.

Soil-water samples were collected and analyzed for oxygen content at a number of irrigation canal sites in northcentral Colorado and the Central Valley of California during the past summer. Prior to field work, the sampling techniques and microanalytical procedures for determining interstitial oxygen in shallow aquatic soil were evaluated in the laboratory. The reliability of the microanalytical technique for determining dissolved oxygen was compared with standard procedures. Some of these data are included in the appendix with the description of the microtechniques.

All field soil-water samples were collected in shallow aquatic soils commonly found in irrigation canal silt bars (not in excess of 6 inches). Normally in canals rooted pondweed species such as sago and American pondweeds are found growing in the shallow soils. Our observations have generally indicated these plants to be shallow rooted in these flowing water situations.

Results of field data obtained during the summer of 1963 are summarized in Tables 22 and 23.

Soil-water oxygen determinations reported in these tables are very preliminary and conclusions regarding any significance to pondweed growth can only be generally discussed at this time. These determinations do, however, show that the sampling device and analytical procedure are useful and can be utilized to make determinations of dissolved oxygen content of interstitial water in canal soils.

It was rather surprising to the authors to find oxygen levels as high as those found in aquatic soil depths tested (1-3 inches) in a saturated silt bar, where most determinations were made. In a saturated soil, especially under static water conditions, one might predict that conditions below the soil-water interface would be quite anaerobic. These determinations suggest a considerable and quite possibly a continuous exchange of soil water or diffusion of dissolved oxygen with the running water of the stream, especially in the less compacted materials.

Table 22

RESULTS OF DISSOLVED OXYGEN DETERMINATIONS IN CANAL WATERS AND INTERSTITIAL WATERS OF THE CANAL BOTTOM SOIL
Samples Obtained from Irrigation Canals in the Vicinity of Fresno, California,
on July 30 and August 1, 1963

Sampling site	Replication No.	Sample type	Observational estimate of canal soil type	Type of pondweed growth in site	Depth soil-water sampl in canal soil (inches)	Water temperature, °F	Dissolved oxygen content (ppm)
Stockton Ditch No. 1, Madera I.D. Sampled on 7/30/63	1	Water	--	American and leafy pondweed	--	62	10 41
	1	Soil	Sandy silt over clay	None	2	--	9.63
	1	Soil	Sandy silt with organic material	In American and leafy weed bank	2-1/2-3	--	7.64
	2	Soil	Sandy silt with organic material	In American and leafy weed bank	2-1/2 3	--	6.90
Stockton Ditch No. 1, Madera I.D. Sampled on 8/1/63	1	Water	--	American and leafy pondweed	--	62	10.37
	2	Water	--	American and leafy pondweed	--	62	9.92
	1	Soil	Sandy silt with organic material	American and leafy weed-bank	1-2	--	5.08
	1	Soil	Sandy silt over clay	None	1-2	--	3.25

Table 22--Continued

Sampling site	Replication No.	Sample type	Observational estimate of canal soil type	Type of pondweed growth in site	Depth soil-water sample in canal soil (inches)	Water temperature, °F	Dissolved oxygen content (ppm)
Kings River Lateral	1	Water	--	Elodea	--	55	10.83
	1	Soil	Sandy with larger gravel	In Elodea bank	1-2	--	10.16
	2	Soil	Some silt over sand-gravel	In Elodea bank	1-2	--	9.0
Stockton Canal, Madera I.D. Sampled on 7/30/63	1	Soil	Organic material mixed in sandy silt	Sparse growth of waterweed	1-2	Same water source as Stockton Ditch	0
	1	Soil	Heavy compacted clay	None	1-2		*8.75
	1	Soil	Looser compacted clay than above	Few American pondweed plants	1-2		*8.83

*Difficulty was encountered in attempting to penetrate this soil with the sampling device. These dissolved oxygen determinations probably reflect surface water because of site disturbance.

Table 23

RESULTS OF DISSOLVED OXYGEN DETERMINATIONS IN CANAL WATERS AND INTERSTITIAL WATERS OF THE CANAL BOTTOM SOIL
Samples obtained from canals in the vicinity of Longmont, Colorado on August 30, 1963

Sampling site	Replication No.	Sample type	Estimate of canal soil type	Type of pondweed growth in site	Depth of soil-water sample in canal soil (inches)	Water temperature, °F	Dissolved oxygen content (ppm)
S. Platte Supply Canal near Canfield, Colorado	1	Water	--	None	--	66	7.17
	2	Water	--	None	--	66	7.50
	1	Soil	Sandy-silt	None	1-1/2	--	1.17
	1	Soil	Sandy-silt	None	1 to 1-1/2	--	3.50
	1	Soil	Sandy-silt	None	2 to 2-1/2	--	2.75
	1	Soil	Sandy-silt	None	2 to 3	--	2.33
S. Platte Supply Canal, 1/2 mile above Canfield, Colorado	1	Soil	Sandy-silt	Leafy pondweed	1-1/2	--	2.67
	1	Soil	Sandy-silt	Leafy pondweed	1-1/2	--	3.85
	2	Soil	Sandy-silt	Leafy pondweed	1-1/2	--	*7.17
	1	Soil	Sandy-silt	Leafy pondweed	3	--	1.83
	2	Soil	Sandy-silt	Leafy pondweed	3	--	1.25
Boulder-Whiterock Canal	1	Water	--	Sago pondweed and algae	--	74	8.58
	1	Soil	Silt with gravel	Clone of sago pondweed	1	--	3.83
	1	Soil	Silt with gravel	Clone of sago pondweed	2		1.50
	1	Soil	Silt-gravel, organic material	Clone of sago pondweed	3-1/2	--	0.0

Table 23--Continued

Sampling site	Replication No.	Sample type	Estimate of canal soil type	Type of pondweed growth in site	Depth of soil-water sample in canal soil (inches)	Water temperature, °F	Dissolved oxygen content (ppm)
Leggett ditch at Highway U.S. 287	1	Water	Gravel-silt, shallow silt bars over compact clay	None	--	72	9.67
	1	Soil	Gravel-silt, shallow silt bars over compact clay	None	1 to 1-1/2	--	8.33
	1	Soil	Gravel-silt, shallow silt bars over compact clay	None	1-1/2 to 2		*9.67

*Samples probably represent oxygen content of water above soil-water interface because of soil disturbance during sampling.

Since compaction of aquatic soils may well be associated with the ability of pondweeds to invade and develop in a canal, measurement of this factor is desirable. Exploration of methods to measure the compaction or looseness of an aquatic soil without disturbance has not been attained by the usual engineering or agricultural methods. Field data obtained to date on soil-water oxygen indicate a possible trend of reduction in the oxygen levels as the soils become finer and more compact. Where large amounts of organic matter occurred in the silt oxygen levels were very low or nil. Probably the micro-organisms decomposing this organic matter were utilizing oxygen at high rates. Further field and laboratory study will be made in an attempt to establish the importance of soil oxygen to growth and development of rooted submersed aquatic weeds that infest irrigation canals.

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APPENDIX

APPENDIX

Methods for Sampling and Determining Dissolved Oxygen Content in Interstitial Water of a Submersed Aquatic Soil

Techniques summarized in this appendix are those evaluated by personnel of the Biological Investigations Section for use in the field determination of soil-water oxygen in canal soils. Various sources of information on techniques were obtained from the literature with the sources cited in the literature reference of the report.

Materials and Methods

Equipment and Methods Used for Interstitial Soil-water Sampling

Eriksen (18) described a water sampling device by which he successfully sampled the soil-water of shallow aquatic substrates. This device was evaluated in initial tests and was found satisfactory for the less compact canal soils. When more compact soils were encountered, the glass constructed device frequently broke so a metal sampler was fabricated from brass tubing. The general dimensions of the unit described below were kept in building the metal model. This device is illustrated in Figure 1. Dimensions are not critical, as long as the reservoir volume is equal to the volume of the sample to be analyzed by micromethods.

The original sampling device was constructed by using a 5-ml volumetric pipette with the upper end of the tube bent at a 45-degree angle. The intake tip was cut off to increase the intake tube diameter. The tip was fitted with a plastic nozzle with holes drilled in the side. The holes were covered with No. 16 bolting silk which was held in place by fine copper wire. The silk acts as a filter preventing entrance of large sand and debris particles. The plastic nozzles used were also fitted with a rubber "O" ring to form a tight seal over the glass pipette. A short piece of surgical rubber tubing, fitted with a pinch clamp, connected to a length of glass tubing is fastened to the other end of the reservoir pipette. The upper glass tube acts as an air vent and the open end must protrude above the water surface.

Methods used in obtaining the soil-water sample were to tighten the pinch clamp at the rubber connecting tube, direct the nozzle upstream and penetrate the substrate nearly parallel to the soil sufficiently far to prevent surface water from following up the tube to the nozzle and contaminating the sample. The vent tube is held above the stream surface and the pinch clamp removed. A pressure difference is created which forces the water in through the nozzle up through the vent pipe. When the pressure difference has equalized and the water level can be seen in the vent tube, the pinch clamp is secured and the sampler removed. The water sample is

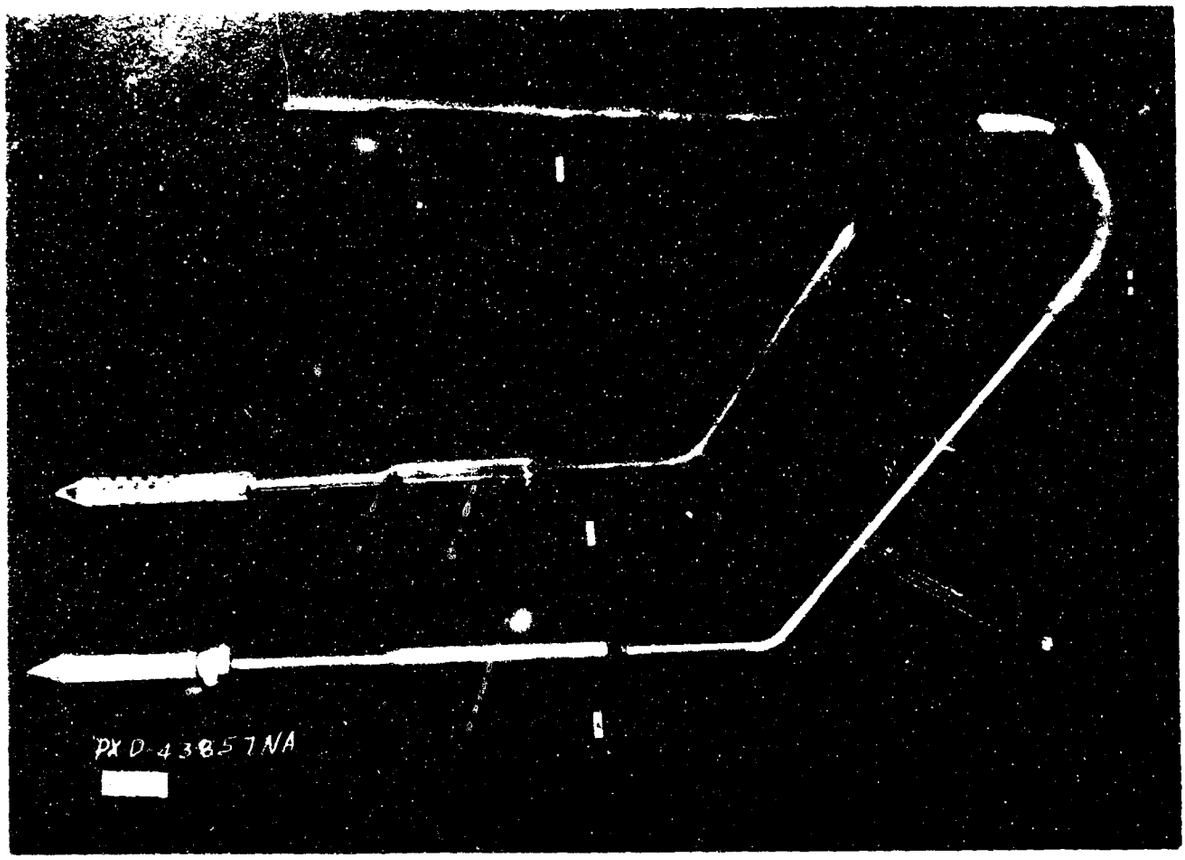

Figure 1. Sampling devices used to obtain aquatic soil interstitial water for dissolved oxygen analyses. The metal unit (A) is constructed from brass tubing for rigidity in sampling in compact soils. Dimensions are similar to the glass sampler (D) described in the text. (B) Surgical tubing and pinch clamp used to seal off water in the sampler. (C) Glass vent tubing.

thus contained in the sampler without further exposure to the air. The first water to enter the sampler is that contacted in the stream. This stream water is forced up above the pinch clamp during sampling of the soil water. The sampled soil water is removed from the 5-ml reservoir at the rubber tubing by use of the hypodermic syringe used in the micro-Winkler chemical determination.

Equipment and Methods Used in the Micro-Winkler Determination of Dissolved Oxygen

Reliable analyses of small water samples obtained by the sampling described above require a precise technique without exposing the water sample to the atmosphere until fixed. Fox and Wingfield (19) described

a suitable technique that was used by Eriksen. Some minor modifications in reagents and equipment were made in our determinations. Basically the analytical method employed is that used in the Winkler procedure for dissolved oxygen determinations. Chemical Laboratory Report No. CH-102 (20) can be referred to for a comprehensive description of dissolved oxygen determinations of canal waters. Procedures for making reagents used in this analysis were for the most part described in this report.

Equipment required for micro-Winkler analyses

Krogh-Keyes syringe pipette, fitted with a standard 5-ml Luer hypodermic syringe and a No. 21 needle. (This device is illustrated in Figure 2.)

Microburette, blue-line, capacity 10 ml, with 0.05-ml graduations.

Miscellaneous equipment such as a burette stand, 50-ml Erlenmeyer titration flasks, distilled water wash bottle, containers for fixing and titration reagents, and small beakers for filling the syringe pipette with reagents.

Reagents for micro-Winkler determinations of dissolved oxygen

1. Manganous sulfate. Dissolve 72.8 grams of $MnSO_4 \cdot H_2O$ in distilled water, filter and dilute to 200 ml.

2. Alkali-iodide-azide. Dissolve 100 grams of NaOH and 30 grams KI in distilled water and dilute to 200 ml. To this solution, add 2 grams of NaN_3 dissolved in 8 ml of distilled water.

3. One percent solution of KI. Dissolve 2 grams of KI (free of iodate) in 100 to 150 ml of distilled water; dilute to 200 ml.

4. Standard sodium thiosulfate stock solution (0.1N). Dissolve 24.82 grams of $Na_2S_2O_3 \cdot 5H_2O$ in boiled and cooled distilled water and dilute to 1 liter. Preserve by adding 1 gram NaOH.

To make 0.001N sodium thiosulfate solution for analysis, use 10 ml of 0.1N solution diluted to 1 liter.

5. Standard potassium biniodate solution (0.025N). Dissolve 3.249 grams of $KH(IO_3)_2$ in distilled water, dilute to 1 liter. Make up necessary 0.001N solution by dilution.

Standardization of sodium thiosulfate solution. In a 50-ml titration vessel add 1 ml of 1-percent KI solution, 2 ml of (0.001N) standard biniodate solution, and 3 drops concentrated phosphoric acid. Add a

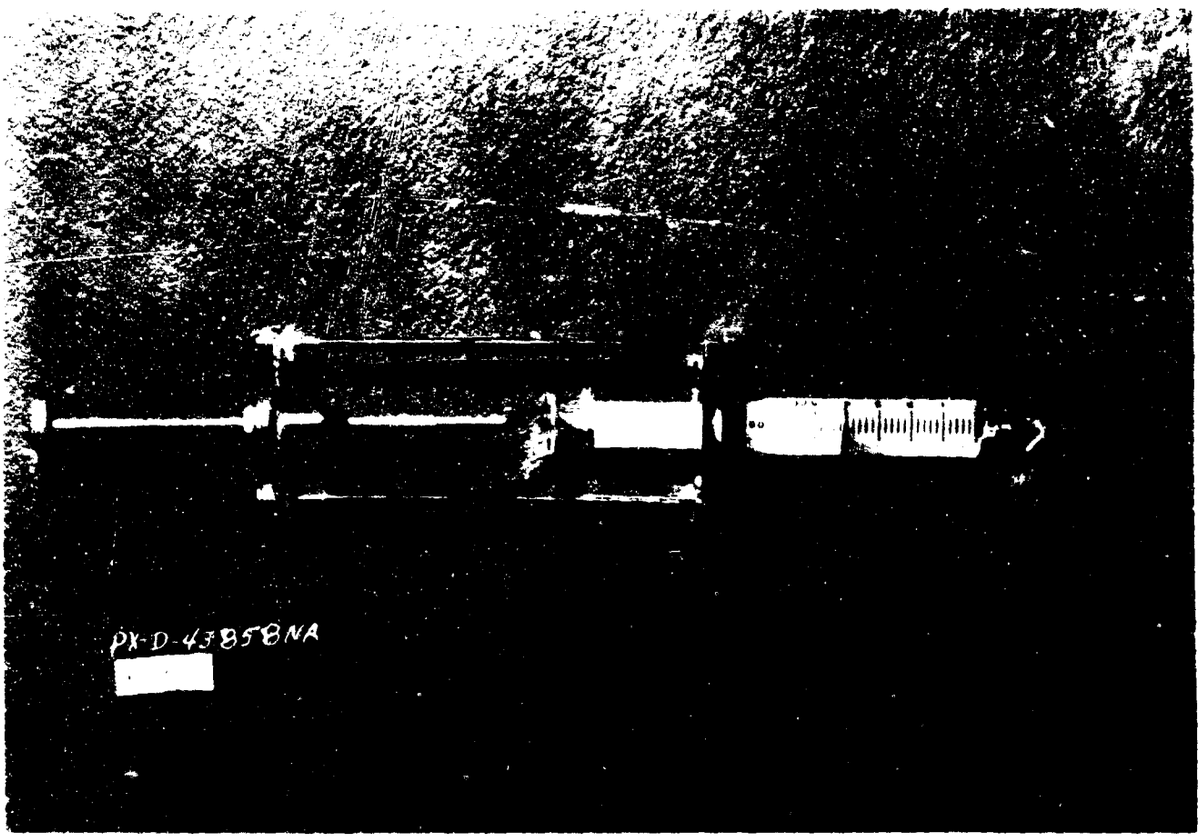

Figure 2. Krogh-Keyes syringe pipette utilized in removing the water sample from the interstitial water sampling device without exposure to air and introducing the fixing reagents of the micro-Winkler analyses for dissolved oxygen in water. The screw stop on the syringe pipette is used to accurately reproduce micro volumes repeatedly.

few milliliters of distilled water to increase volume. Titrate to a clear end point with ±2 ml of standardized (0.001N) thiosulfate solution using starch solution as indicator. Sodium thiosulfate solution is somewhat unstable and must be made up fresh occasionally. The standard 0.001N solution should be restandardized prior to each field test series.

Calculate normality of the sodium thiosulfate solution as follows:

$$\text{Normality} = \text{Calculated normality of thiosulfate solution} \times \frac{\text{ml of biniodate solution x 100}}{\text{ml of thiosulfate solution used x 100 to reach endpoint in titration}}$$

Example: $N = 0.001 \times \frac{200}{200} = 0.001$

6. Starch indicator. Prepare a thin paste of about 2 grams of soluble starch in cold water. Pour into 200 ml of boiling water and boil for a few minutes. Cool and add a few drops of toluene for preservative.

7. Phosphoric acid. Concentrated USP 85 percent was found to be satisfactory. Standard Winkler procedures use sulfuric acid, but this acid will corrode the metal parts on the pipette syringe.

Calibration of the volume of the syringe pipette and dead space in the syringe needle

Working volumes of the syringe pipette must be known precisely for calculations of dissolved oxygen reacted. This must be done chemically, because of precision required and microvolumes involved.

1. Determination of total volume of syringe barrel plus dead nozzle (syringe needle) space. Add 1 ml 1-percent KI solution and 3 drops phosphoric acid to titration vessel (50 ml Erlenmeyer flask). Fill the 5-ml Krogh-Keyes syringe pipette to capacity, approximately 5 ml, with 0.001N biniodate solution by backing stop screw up until the plunger contacts it at the 5-cc volume index on the syringe. Set the locknut on the stop screw securely at this point. Discharge biniodate solution into titration vessel containing KI solution and phosphoric acid. Rinse syringe barrel twice with distilled water, pulling a full volume of water into the syringe each time. Titrate with standardized 0.001N sodium thiosulfate solution to a clear end point, using starch indicator. Calculate total volume of syringe pipette as follows:

Total volume (barrel + dead space) =

$$\text{Amount of thiosulfate used in titration} \times \frac{\text{ml of biniodate solution used in standardization}}{\text{ml of thiosulfate solution used in standardization}}$$

Example:

$$\text{Total volume of syringe} = 4.58 \text{ ml} \times \frac{200}{200} = 4.58 \times 1 = 4.58 \text{ ml}$$

2. Determination of dead space volume of syringe needle. Thoroughly rinse needle with distilled water by sucking water into the syringe and discharging. Fill syringe needle with excess of biniodate solution (0.001N) and invert syringe and expel excess solution by full travel of syringe plunger. Rinse outside of the needle with distilled water. Pick up bioniodate solution contained in needle by pulling a few milliliters of distilled water into the syringe. Discharge this into a vessel and titrate as before.

Calculate dead space volume of syringe needle as follows:

$$\text{Dead space volume of needle} = \text{Amount of thiosulfate used in titration} \times \frac{\text{ml of biniodate solution used in standardization}}{\text{ml of thiosulfate solution used in standardization}}$$

Example:

$$\text{Dead space volume} = 0.15 \text{ ml} \times \frac{200}{200} = 0.15 \times 1 = 0.15 \text{ ml}$$

$$\text{Volume of syringe used in determining dissolved oxygen content} = \text{Total volume of syringe (barrel + dead space) minus volume of dead space in needle}$$

Example: 4.58-0.14 = 4.43 ml (working volume of syringe)

Procedures for Determination of Dissolved Oxygen Contained in Interstitial Water Sample or Stream Sample

1. Add $MnSO_4$ solution by pulling an excess into the syringe pipette, invert and discharge the excess. Rinse outside of needle with distilled water. (This amount will be that volume previously determined as the dead space in the pipette syringe.)

2. Pick up the water to be analyzed for dissolved oxygen content by carefully inserting the syringe needle into the rubber tubing of the water sampler. Water must be obtained below the pinch clamp. Stream water can also be easily analyzed by this technique utilizing the soil-water sampler. Care must be exercised in pulling water into the syringe so not to introduce air bubbles. Move syringe plunger until it contacts the stop screw at the predetermined and set volume of 5 ml.

3. Alkaline KI-azide solution is next drawn into the syringe by backing off the screw stop one turn from the 5 ml set position. This volume should be equal to about twice the volume of the dead space. (This was determined by the same procedures previously described for calibration of the dead space volume of syringe.)

4. The syringe pipette is shaken a few times until the precipitate of manganous hydroxide is evenly distributed throughout the syringe. The pipette is allowed to lay on its side for about 3 minutes for complete absorption of dissolved oxygen by the precipitate. It was found that the needle must be plugged during the various fixing procedures to prevent leakage of the reaction components and the sample. Insertion of the needle into the wall of surgical tubing worked well.

5. The stop screw is backed off an additional three turns after further mild agitation of the syringe. Phosphoric acid is drawn into the syringe until the plunger contacts the stop screw. The unit is shaken by a rocking motion until all of the milky white precipitate has dissolved and iodine is liberated. This solution is then discharged into a 50-ml Erlenmeyer flask for titration of iodine. A few milliliters of distilled water are added to increase volume prior to 4-5 drops of the starch indicator.

6. Titrate this unknown (using the 10-ml blue-line burette) with standardized 0.001N sodium thiosulfate to a clear end point. It has been found that the solution is light straw color at the start of the titration procedure so the starch indicator must be added at the start. Unlike normal titrations of liberated iodine by thiosulfate methods where the starch indicator is added when the titration nears the end point.

Calculate dissolved oxygen content of water sample as follows:

$$\text{Dissolved oxygen, in parts per million (ppm)} = \frac{\text{ml of thiosulfate used in final titration} \times \text{N of thiosulfate solution} \times 8 \times 1000}{\text{Working volume of the syringe pipette as determined}}$$

Example:

$$\frac{4.36 \text{ ml} \times 0.001\text{N} \times 8 \times 1000}{4.43 \text{ ml}} = 7.91 \text{ ppm of dissolved oxygen}$$

Microanalytical techniques are often unreliable and difficult to obtain reproducible results. To ascertain the reliability of the micro-Winkler technique and manipulations by the investigators, water samples were

simultaneously analyzed by both the micro and the usual standard Winkler procedure for dissolved oxygen. The standard Winkler procedures were followed as described in Chemical Laboratory Report No. CH-103 as cited from Standard Method for the Examination of Water and Waste Water (20).

Tapwater was used for a representative sample and always contained dissolved oxygen at saturation values, as corrected for temperature and altitude.

Results of some of these comparative tests are given in Table 1.

Table 1

REPRESENTATIVE COMPARISONS OF THE STANDARD WINKLER METHOD WITH THE MICRO-WINKLER METHOD IN THE ANALYSES OF DISSOLVED OXYGEN CONTENT OF TAPWATER

Analysis No.	Water temperature, °C	Dissolved oxygen content, ppm	
		Micro-Winkler method	Standard Winkler method
1	19	7.30	7.48
2	19	7.25	7.38
3	18	7.25	7.38
4	15	8.30	8.41
5	15	8.32	8.41

These representative comparisons indicate that the micro-Winkler procedure is well within the 2-percent limits of the more commonly used macro procedure, as indicated by previous investigators (17) (18).